Community genetics of eucalypts: Provenance effects on canopy communities, potential drivers and underlying QTL

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A thesis submitted in fulfillment of the requirements for a degree of
Doctor of Philosophy

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February, 2017

Declarations

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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Statement of Publication and Contribution

Chapter 2 is in preparation for submission for peer-review publication as:

Gosney BJ, O'Reilly-Wapstra JM, Forster LG, Whiteley C, Potts BM (in prep) Stability of community genetic effects across space and time: Insights from *Eucalyptus*.

The candidate was the lead author of this chapter and was responsible for the analysis of the data, the development of ideas and writing of the manuscript. The candidate, BMP, JMO and LGF contributed to the development of the experimental methodology for the *E. pauciflora* portion of the study. The candidate was responsible for the arthropod and fungal community assessment for the *E. pauciflora* portion of the study. BMP, JMO, LGF and CW contributed to the development of the experimental methodology for the *E. globulus* portion of the study. CW was responsible for the arthropod and fungal community assessment for the *E. globulus* portion of the study. LFG assisted with the identification and refining of arthropod and fungal symptoms for both portions of the study. BMP provided guidance for data analysis. BMP and JMO provided guidance for the development of ideas and writing of the manuscript. BMP and JMO assisted with the refining of the manuscript.

Chapter 3 has been published as:

Gosney BJ, O'Reilly-Wapstra JM, Forster LG, Barbour RC, Iason GR, Potts BM (2014) Genetic and ontogenetic variation in an endangered tree structures dependent arthropod and fungal communities. *PLoS ONE* 9(12): e114132. doi:10.1371/journal.pone.0114132.

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Chapter 4 has been published as:

Gosney BJ, Potts BM, O'Reilly-Wapstra JM, Vaillancourt RE, Fitzgerald H, Davies NW, Freeman JS (2016) Genetic control of cuticular wax compounds in *Eucalyptus globulus*. *New Phytologist* 209(1), 202-215. doi: 10.1111/nph.13600.

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Chapter 5 has been submitted to a peer-reviewed journal as:

Gosney BJ, O'Reilly-Wapstra JM, Forster LG, Whiteley C, Potts BM (submitted) The extended community-level effects of genetic variation in foliar wax chemistry in the forest tree, *Eucalyptus globulus*.

The candidate was the lead author of this chapter and was responsible for analysis of the data, the development of ideas and writing of the manuscript. BMP, JMO, LGF, and CW contributed to the development of the experimental methodology. CW was responsible for the arthropod and fungal community assessment. LGF assisted with the identification of refining of the arthropod and fungal symptoms. BMP provided guidance for data analysis. BMP and JMO provided guidance for the development of ideas and writing of the manuscript. BMP, JMO, LFG, and LGW assisted with the refining of the manuscript.

Chapter 6 is in preparation for submission for peer-review publication as:

Gosney BJ, Potts BM, Vaillancourt RE, O'Reilly-Wapstra JM, Barbour RC, Forster LG, Freeman JS (in prep) Bioactive QTL and evidence for emergent genetic effects on the dependent arthropod and fungal community of a forest tree.

The candidate was the lead author of this chapter and was responsible for the analysis of the data, the development of ideas and writing of the manuscript. JSF, BMP, REV, JMO, LGF and RCB contributed to the development of the experimental methodology. LGF was responsible for the assessment, identification and refining of the arthropod and fungal community. JSF provided guidance for data analysis. JSF, BMP, REV and JMO provided guidance for the development of ideas and writing of the manuscript. JSF, BMP, REV and JOR assisted with the refining of the manuscript.

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Abstract

Genetic variation in foundation trees can have an extended effect on their dependent communities. Extended genetic effects have been shown to influence the abundance, richness and composition of canopy communities in many forest tree systems. However, few community genetic studies have been undertaken on southern hemisphere trees. This thesis focuses on the important genus *Eucalyptus*. Trees of this genus dominate much of Australia's native forests and woodlands but are grown globally for forestry and other uses. Eucalypt species are genetically diverse and it is important to understand the influence of this genetic diversity on dependent biodiversity. This is particularly so for large-scale forestry and restoration plantings where there is increasing interest in the use of non-local provenances as a response to global climate change, but is also an issue for native forest management and conservation.

Three well-studied eucalypt systems – *E. globulus*, *E. morrisbyi* and *E. pauciflora* – were used to study the relative importance of provenance variation in shaping the canopy community of dependent arthropod and fungi, potential mechanisms driving genetic-based differences and underlying regions of the genome impacting on the dependent community. The thesis specifically aimed to determine: 1) the relative importance of extended genetic effects in comparison to known factors influencing biotic communities, including site, yearly and ontogenetic effects; 2) the stability of extended genetic effects across environments and years to better understand the potential extended consequences of provenance translocation in forestry, restoration and conservation; 3) the relative importance of foliar chemicals as potential drivers of genetic-based variation in dependent community responses in *E. globulus*; and 4) the regions of the genome impacting on dependent community responses.

Significant variation in the dependent canopy community among provenances was detected following natural colonization of common garden field trials of all three eucalypt systems. The relative influence of genetic-based effects on dependent community composition was comparable to that of ontogenetic effects (*E. morrisbyi*), while site (*E. globulus* and *E. pauciflora*) and yearly effects (*E. pauciflora*) far outweighed that of genetic-based effects. Nevertheless, broad trends in genetic-based community variation among provenances were detected across sites and years and associated with reported patterns of adaptive differentiation within the focal tree species associated

with latitude (*E. globulus*) and altitude (*E. pauciflora*). The association of genetic-based variation in physiochemical properties of leaves on the dependent community of *E. morrisbyi* and *E. globulus* provided insights into potential foliar chemical mechanisms driving these genetic-based community differences. Investigation of the cuticular waxes of *E. globulus* showed significant genetic control and differences among provenances, with signals of divergent selection in four of the thirteen quantified wax compounds. These four cuticular wax compounds along with seven foliar terpenes explained a combined 34% of the provenance differences in canopy community composition. Overall the influence of foliar wax compounds was comparable to that of the well-studied foliar terpenes. However, while quantitative trait loci (QTL) for community traits were detected in the mapping family studied, none of the chemical compounds associated with provenance differences in the canopy community were associated with these genomic regions. Furthermore, while many QTL were detected for individual community members, and some of these co-located with QTL for foliar chemicals, none co-located with QTL for community traits.

The work presented in this thesis advances the field of community genetics in several ways. Extended genetic effects have been detected in numerous plants systems, however, the partitioning of variation to determine the relative importance of genetic effects and potential mechanisms is rare. While this thesis shows that extended genetic effects in eucalypts may be small in comparison to effects such as site and year, it provides evidence that adaptive variation in phenotypic traits can have extended effects on communities and ecosystem processes, and this influence appears consistent across sites and years. Additionally, it highlights the complexity underlying both phenotypic and genetic-based variation in dependent canopy communities with QTL providing evidence of community-level emergent genetic effects.

Acknowledgments

I would like to thank my supervisors Brad Potts, Julianne O'Reilly-Wapstra and Jules Freeman, as well as René Vaillancourt whose expertise, guidance and support made the work in this thesis possible. I very much enjoyed our discussions and working with all of you.

I would like to express my gratitude to Lynne Forster whose kindness, enthusiasm, expertise and contribution were invaluable over the course of the work in this thesis.

I would like to thank all the people involved in collecting and allowing me to work on the numerous data sets in this thesis. Special thanks go to Carmen Whiteley, Bob Barbour, Hugh Fitzgerald, Noel Davies and Glenn Iason.

I am thankful to Peter Harrison whose friendship over the past few years has kept me sane while living half a world away from home.

I would also like to thank John Senior and Stuart McDonald for being great office mates.

Thank you to my Mt. Nelson family, Matt Larcombe, Rebecca McWatters and Phaedra Brothers for all the fun times of food, drink and conversation. Special thanks to my fellow North American, Rebecca, who ensured that I was not homeless for my last few month in Tasmania.

Finally, I am forever grateful to my family, whose confidence, support and love cannot be replaced.

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Chapter 1: Introduction

1.1 Community genetics

Community and ecosystem genetics is defined as "the study of the genetic interactions that occur between species and their abiotic environment in complex communities" (Whitham *et al.* 2006). The field of community genetics aims to discern whether individual genes have extended effects outside the individual, influencing local community and ecosystem processes. Most of the work in this field has been centered around the influence of genetic variation among and within plant species with primary focus on trees (Whitham *et al.* 2010), while also studied in forbs (Agrawal 2005), grasses (Vandegehuchte *et al.* 2011), and shrubs (Barbour *et al.* 2015). Foundation species, such as forest trees, have a disproportionate influence on their surrounding environment, creating locally stable conditions for the colonization of organisms, development of communities and operation of ecosystem processes (Ellison *et al.* 2005), making them highly relevant to the study of extended genetic effects (Whitham *et al.* 2006).

Previous studies in community genetics show that genetic variation in forest trees can have extended effects on dependent communities and ecosystems processes (Whitham *et al.* 2012). These extended genetic effects can influence multiple ecosystem levels from foliar herbivores and pathogens (Bangert *et al.* 2006b; Barbour *et al.* 2009c), under-bark invertebrates (Barbour *et al.* 2009b), below-ground microbes (Schweitzer *et al.* 2008a), to higher trophic interactions (Bailey *et al.* 2006). They can impact on individual organism abundances and community parameter, such as total organism abundance, organism richness and diversity measures, as well as overall community composition (Barbour *et al.* 2009c; Crutsinger *et al.* 2006; Maddox and Root 1987; Whitham *et al.* 2006; Wimp *et al.* 2007). These extended genetic effects can take place at multiple genetic scales including among clones (Fritz and Price 1988; Korkama *et al.* 2006; Robinson *et al.* 2012; Silfver *et al.* 2014; Whitham *et al.* 2006), families (Axelsson *et al.* 2015; Roche and Fritz 1997), and provenances (Barbour *et al.* 2009c; Sinclair *et al.* 2015). Further, specific quantitative trait loci (QTL) (DeWoody *et al.* 2013; Rönnberg-Wästljung *et al.* 2006) and gene (Zinkgraf *et al.* 2016) effects have been detected. In addition, a lot of the early community genetics studies were

focused on plant hybrids, a scale which included interspecific differences among hybridizing species (Bangert *et al.* 2006b; Dungey *et al.* 2000; Floate *et al.* 1993; Hochwender and Fritz 2004; Wimp *et al.* 2007). While extended genetic effects have been established in many plant species, numerous gaps in our knowledge and understanding of such effects still exist (Crutsinger 2016; Hersch-Green *et al.* 2011). Few studies in community genetics have examined the potential biotic consequences to an increasing popularity in provenance translocation practices, the potential mechanisms driving genetic-based community differences, and the underlying genomic regions impacting on community-level responses.

1.2 Provenance variation in forest trees

Most plant species display a geographic pattern in genetically-based traits (Hamrick 1989; Morgenstern 2011). In forest trees, geographic patterns of genetic-based variation have been a topic of study for the better part of two centuries. Such patterns are due to local adaptations and genetic drift and are often associated with environmental gradients within forest tree species, such as latitudinal and altitudinal clines (Morgenstern 2011; Mousseau *et al.* 2000). Environmental variation along these gradients, such as soil, temperature and rainfall differentiations, drive adaptive divergence within species and can be displayed as numerous phenotypic traits (Kremer *et al.* 2014; Morgenstern 2011; Mousseau *et al.* 2000). For example, substantial increases in light-saturated photosynthesis, stomatal conductance, foliar nitrogen and leaf mass has been observed with increasing altitudes in both the European beech *Fagus sylvatica* and the sessile oak *Quercus petraea* (Bresson *et al.* 2011). The use of common garden trials to study geographic trends in phenotypic traits has allowed for determination of their genetic basis (Mousseau *et al.* 2000). These trials are designed to eliminate as much of the environmental variation as possible with seed collected across the geographic range of the species planted in a randomized single field trial; thus maximizing the detection of genetic-based differences. In Bresson *et al.* (2011) , a genetic basis to observed phenotypic differences was detected for leaf mass in *Fagus sylvatica* and foliar nitrogen content in *Quercus petraea* from a common garden trial, indicating that observed differences in light-saturated photosynthesis and stomatal conductance were primarily driven by the environment (Bresson *et al.* 2011).

Understanding of genetic-based provenance difference in traits such as tree growth rates, survival and other fitness related traits could substantially improve results in commercial, conservation and restoration practices. In the case of restoration, a 'local is best' approach has been regarded as optimal as local provenances are likely adapted to local environmental conditions and are thus more suitable then non-local provenances (Broadhurst *et al.* 2008). However, the use of non-local provenances (translocation) is increasingly being promoted as a response to a globally changing climate for commercial, conservation and restoration practices (Aitken and Whitlock 2013; Breed *et al.* 2013; Gray *et al.* 2016; Prober *et al.* 2016). While the importance of provenance variation in forest trees is evident, little is known of their extended genetic effects and the potential biotic consequences to provenance translocations (Bucharova 2016; Frascaria-Lacoste and Fernandez-Manjarres 2012). Such translocations could result in the alteration of the evolutionary trajectory of local biotic systems where plantings occur. For example, local adaptation of genotypes may provide resistance to major pest species not present in non-local genotypes (Endler *et al.* 2010). Alternatively, planting of non-local genotypes may result in the loss of organisms from the biotic community due to their specific adaptation to local plant genotypes. However, the importance of these extended genetic effects in regard to provenance translocations in commercial, conservation and restoration practices depends on their relative magnitude and stability across environments and over time. Understanding the relative magnitude and stability of genetic-based provenance effects on the dependent community across environment and time can provide insight into the potential consequences of provenance translocations.

1.3 Potential mechanisms driving extended genetic effects

Few studies have addressed the genetic-based mechanisms driving patterns of provenance variation in canopy communities. Many genetic-based traits are likely to influence community responses to provenance variation. Previous studies have shown significant associations of the biotic community with genetic-based variation in tree morphological (Barbour *et al.* 2015; Barbour *et al.* 2009c; Robinson *et al.* 2012), architectural (Steinbauer *et al.* 1998) and chemical (Barbour *et al.* 2009c; Jones *et al.* 2002; Whitham *et al.* 2006) traits. For example, provenance variation in the dependent canopy community of the forest tree *Eucalyptus globulus* has been associated with provenance differences in specific leaf dry mass, lamina length, condensed tannins and the formylated phloroglucinol compound (FPC) macrocarpal G (Barbour *et al.* 2009c). This same

study also showed significant association of provenance differences in canopy community with overall physiochemical profile (near-infrared spectroscopy) and morphology, indicating that communities may respond to not just individual traits but their combination and interactions on a multivariate level (Barbour *et al.* 2009c). Additionally, provenance variation in canopy communities may be driven in part by indirect genetic effects through ecological interactions between organisms (Mooney and Agrawal 2008; Wolf *et al.* 1998). While many genetic-based traits may be influencing provenance variation in canopy communities, phytochemistry has long been implicated as a key mechanism driving these extended genetic effects.

Of the potential chemical mechanisms driving extended genetic effect, condensed tannins are perhaps the most widely studied. Condensed tannins are the most abundant secondary metabolites produced by plants and are historically shown to influence the feeding of many herbivore species (Barbehenn and Constabel 2011; Bernays 1981; Kraus *et al.* 2003). In addition to their potential bioactivity, condensed tannins have also been implicated in nutrient dynamics of forest ecosystems including nitrogen mineralization (Norris *et al.* 2011), microbial activity (Schimel *et al.* 1996) and nutrient retention (Sivapalan 1981). Studies in community genetics have supported the potential bioactivity of variation in condensed tannins. Differences in phenotypic variation in condensed tannins among the forest trees *Populus angustifolia*, *Populus fremontii* and their F_1 and backcross hybrids were significantly associated with terrestrial, endophyte and aquatic community-level responses (Whitham *et al.* 2006). Similarly, provenance differences in condensed tannins in *Eucalyptus globulus* were also shown to be associated with differences in the dependent arthropod and fungal community (Barbour *et al.* 2009c), suggesting a potential chemical mechanistic driver.

Other secondary metabolites including FPCs, terpenes and waxes may also be driving extended genetic effects. In *Eucalyptus*, FPCs are considered bioactive with genetic-based variation in FPCs considered the primary component determining marsupial herbivory (Moore *et al.* 2004; O'Reilly-Wapstra *et al.* 2010; Wallis *et al.* 2002), as well as showing an impact on insect herbivores (Andrew *et al.* 2007). Additionally, these FPCs have shown strong genetic control in eucalypts with identification of quantitative trait loci in both *E. globulus* (Freeman *et al.* 2008a) and *E. nitens* (Henery *et al.* 2007), as well as provenance effects in *E. globulus* (O'Reilly-Wapstra *et al.* 2013b). Similarly, QTL for foliar terpenes have also been identified in both *E. globulus* (O'Reilly-Wapstra

et al. 2011) and *E. nitens* (Henery et al. 2007) and have been implicated as defensive compounds to mammalian herbivory in *E. globulus* (O'Reilly-Wapstra *et al.* 2004; Padovan *et al.* 2013), as well as showing significant provenance effects (O'Reilly-Wapstra *et al.* 2013b). While extended genetic effects of foliar terpenes to the dependent canopy community have not been examined in eucalypts, their influence is apparent with many other studies showing a strong association with insect herbivores (Bustos-Segura *et al.* 2015; Stone and Bacon 1994).

Cuticular wax compounds are perhaps the least studied of the secondary metabolites in relation to their possible extended genetic effects. They are the primary barrier of a plant to its environment, providing protection from water loss, ultraviolet rays, insect herbivores and pathogens (Riederer and Schreiber 1995; Tucker *et al.* 2010; Yeats and Rose 2013). Clinal variation in wax phenotypes has been observed along environmental gradients in many forest trees indicating a functional importance (Barber 1955; Hamrick 1976). Unlike the other secondary metabolites, cuticular waxes can affect organism through both structural and chemical mechanisms, with evidence of insect herbivore resistant phenotypes (Bodnaryk 1992; Eigenbrode and Espelie 1995) and an inability of organisms to adhere to waxy surfaces (Edwards 1982; Eigenbrode 2004). In eucalypts, cuticular wax compounds vary between species (Li *et al.* 1997) and have shown evidence of possible bioactivity in relation to susceptibility to defoliation by an arthropod pest species (Jones *et al.* 2002). Despite their importance and evidence of an extended genetic effect, little is known of the genetic-based variation and control of cuticular waxes in forest trees and the extended genetic effects of these compounds are poorly understood. Additionally, studies of the influence of phytochemistry on the dependent community have primarily focused on showing a significant association and the relative magnitude of their effects are rarely examined.

1.4 Genomic regions underlying extended effects

Quantitative trait loci (QTL) analysis is a widely used and accepted method for identifying the underlying regions of the genome associated with phenotypic variation in quantitative traits and are often used as a precursor for identification of the genes impacting on the trait of interest (Mackay *et al.* 2009; Mauricio 2001). In forest trees, QTL studies have been undertaken on traits such as growth, wood properties (Bradshaw and Stettler 1995; Freeman *et al.* 2009; Grattapaglia *et al.* 2009), drought (Hamanishi and Campbell 2011; Rönnberg-Wästljung *et al.* 2005; Street *et*

al. 2006) and frost (Byrne *et al.* 1997; Tsarouhas *et al.* 2004) tolerance. Previous studies of potential bioactive QTL in forest trees have focused on individual organism responses, particularly that of major pest organisms. In *E. globulus* F_2 mapping populations, QTL have been identified for resistance to multiple fungal pathogens (Butler *et al.* 2016; Freeman *et al.* 2008b) as well as showing evidence of resistance to autumn gum moth (*Mnesampela privata*) defoliation (Jones *et al.* 2002). Whether these QTL effects on individual organisms extend to dependent community responses is poorly understood.

Few studies have examined the genomic regions underlying extended genetic effects on dependent canopy communities in forest trees. Examining these genomic regions could allow for identification of individual genes, alleles and potential underlying mechanisms driving dependent community responses to further our understanding of the evolutionary processes that shape biotic interactions (Whitham *et al.* 2008). In a *Salix dasyclados* × *S. viminalis* tetraploid hybrid F_2 population, QTL were identified for independent damage type abundances (i.e. feeding guild damage, each associated with numerous causal organisms) at three different field sites (Rönnberg-Wästljung *et al.* 2006). Few QTL in the Rönnberg-Wästljung *et al.* (2006) study were consistent among common garden trials indicating a potential interaction of QTL with the environment. Additionally, most identified QTL in this study were specific to an individual damage type with few cases of QTL for multiple damage types. The few cases were hypothesized by the authors to be underlain by genes controlling defensive compounds (Rönnberg-Wästljung *et al.* 2006), however, causal mechanisms were not examined in this study. Evidence of potential genomic regions for defensive compounds having extended effects on community-level responses was provided by a study of a *Populus trichocarpa* × *P. deltoids* hybrid F_2 population. Numerous QTL for insect damage types (i.e. chewer, skeletonizer, leaf miner, gall former, leaf rollers and sap suckers) were shown to co-locate with reported phenolic pathway genes, while also showing co-location of a few QTL for damage types with QTL for leaf morphological traits (DeWoody *et al.* 2013). While both the Rönnberg-Wästljung *et al.* (2006) and DeWoody *et al.* (2013) study provide insights into extended genomic effects in forest trees, no studies have examined whether regions of the genome influencing individual organisms and guild responses extend to overall community-level effects, such as total organism abundance, richness and composition.

1.5 Study systems

The tree genus *Eucalyptus* is part of the family *Myrtaceae* and is the dominant tree system in Australia with nearly 900 identified taxa (Centre for Plant Biodiversity Research and Slee 2006). This thesis explores the extended genetic effects of three of these taxa, *E. morrisbyi*, *E. pauciflora* and *E. globulus*, that combined provide a broad view of provenance impacts on developing canopy communities in eucalypts.

1.5.1 Eucalyptus morrisbyi

Eucalyptus morrisbyi, commonly known as Morrisbys gum, is an endangered eucalypt species native to the island of Tasmania, Australia and represents a species of conservation value in this thesis. The species is part of the subgenus *Symphyomyrtus* and occurs naturally in coastal, dry sclerophyll woodlands with poor drainage (Threatened Species Section 2009; Williams and Potts 1996). Like many eucalypt species, *E. morrisbyi* is heteroblastic whereby leaves of the tree change markedly from juvenile to adult developmental stages (Wiltshire *et al.* 1998; Zotz *et al.* 2011). Juvenile leaves of *E. morrisbyi* are glaucous, unstalked, arranged opposite and are about 2-3cm long and 2-4cm wide, while adult leaves are stalked, more green, arranged alternately and are about 5-10cm long and 1.5-4cm wide (Threatened Species Section 2009). Mature trees are relatively small ranging from 6-12m while also exhibiting a mallee form. Mallee individuals regenerate from the basal lignotubers of the tree after the death of the main stem, which usually occurs due to instances of fire and drought (Jones *et al.* 2005).

The tree species is restricted to four populations with two main populations (Risdon Hills and Calverts Hill; Fig. 1.1) and two small populations (Lumeah Point and Honeywood Drive) consisting of only a few individuals (Jones *et al.* 2005). The Risdon Hill population (42°49’S, 172°20’E) is the smaller of the two main populations consisting of 81 mature individuals and is separated by nearly 20km from the larger Calverts Hill population (42°56’S, 147°31’E) consisting of 1915 mature individuals (Jones *et al.* 2005). All individuals in the Risdon Hill population are in the mallee form and are in declining health due to drought and heavy insect damage while up to a decade ago the Calverts Hill population was relatively healthy with many sizable individuals. However, since then there has been over 90% mortality of mature trees at Calverts Hill (Jones *et al.* 2016). Investigation of the genetic variation within the species has shown substantial

differentiation between the two main populations with little to no sharing of chloroplast haplotypes and microsatellite alleles (Jones *et al.* 2005). Additionally, microsatellites show relatively high genetic diversity within both the Risdon Hill and Calverts Hill populations and little differences in inbreeding levels between the populations (Jones *et al.* 2005). Combined, the high genetic diversity between and within the two main populations of *E. morrisbyi* indicate the importance of the populations in maintaining the diversity of the species in conservation efforts. *In situ* conservation efforts for the two main populations has been implemented since 1992 (Blackhall and Lynch 1992). Seed has been collected for long term conservation storage and *ex situ* conservation plantings have been established and maintained by Forestry Tasmania and the University of Tasmania (Threatened Species Section 2009).

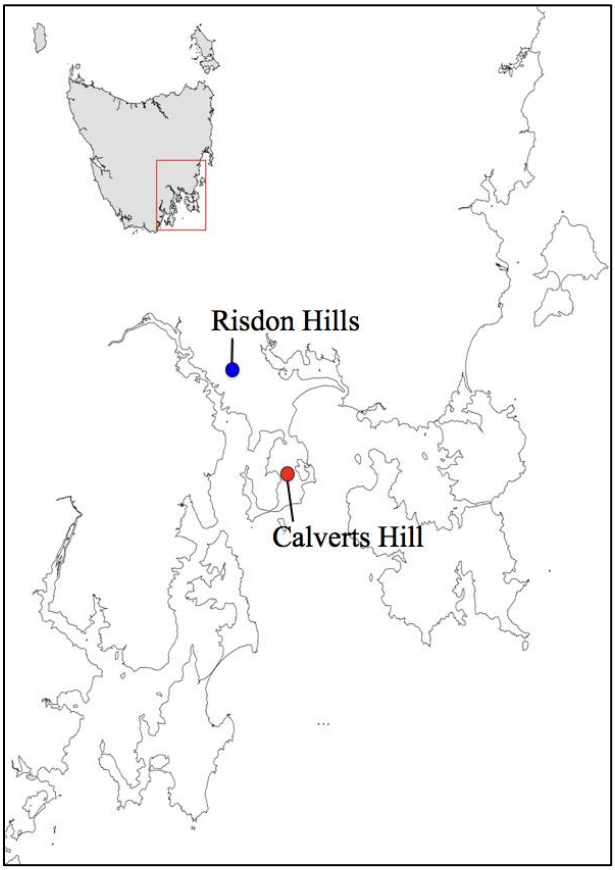

Fig. 1.1. Geographic locations of the two main populations of *Eucalyptus morrisbyi* in Tasmania.

Considering the conservation status and high genetic diversity in *E. morrisbyi*, understanding the extended effects of genetic variation in the species would allow for improved management of the populations (Hersch-Green *et al.* 2011; Whitham *et al.* 2010). While particularly relevant in *E. morrisbyi* due to ongoing *in situ* and *ex situ* conservation efforts, little is known of the extended genetic effects in the species. To date, Mann *et al.* (2012) is the only study to address such effects with significant differences between the main population of *E. morrisbyi* in the susceptibility to browsing by the marsupial herbivore *Trichosurus vulpecula*, commonly known as the brushtail possum. The Calverts Hill population was the more susceptible to damage than the Risdon Hill population resulting in poor tree growth and performance (Mann *et al.* 2012). Additionally, captive feeding trials indicate that differences in browsing susceptibility between populations may be due in part to differences in morphological and chemical traits with the less browsed Risdon Hill population exhibiting thicker leaves and higher levels of the formylated phloroglucinols (FPC) sideroxylonal A and C (Mann *et al.* 2012). While Mann *et al.* (2012) suggested that genetic-based variation in phenotypic traits can have extended effects to the biotic environment in *E. morrisbyi*, further investigation of the range of these extended genetic effects to other levels, such as the arthropod and fungal canopy community, would allow for better management, not just of the populations of *E. morrisbyi*, but also their dependent communities. This is particularly important as translocation of seed from the Risdon Hill population has recently been discussed as a management option to help restore the dramatically declining Calverts Hill population (Jones *et al.* 2016).

1.5.2 Eucalyptus pauciflora

Eucalyptus pauciflora is a heteroblastic tree, commonly known as the snow gum, native to Tasmania and south-eastern Australia and represents a species of restoration value in this thesis. The species is part of the subgenus *Eucalyptus* (*Monocalyptus*) and occurs naturally across a wide range of environments ranging in altitude from just above sea level to nearly 2000m (Boland *et al.* 2006; Williams and Potts 1996). Trees predominately occur in dry sub-alpine habitats on well drained soils and range in size from 6m at higher altitudes to 30m at lower altitudes (Boland *et al.* 2006; Nicolle 2006). Genetic-based clinal variation with altitude has been long-known in this species (Pryor 1956). For example, altitudinal clines in *E. pauciflora* have been reported in tree form and bark thickness (Pryor 1956), photosynthetic traits (Slayter and Morrow 1977) and frost

tolerance (Green 1969). *E. pauciflora* is one of the most widespread eucalypt species in Australia with an ability to survive a wide array of climates with a mean annual temperature ranging from 4.1°C to 15.4°C and a mean annual rainfall ranging from 600mm to 1900mm (Boland *et al.* 2006). Additionally, this species is known to survive extreme environmental conditions, such as periodic droughts (Boland *et al.* 2006; Williams and Potts 1996), which makes it an ideal species for forest restoration practices in a changing climate.

Until recently, knowledge of the genetic-based variation within *E. pauciflora* has been based on studies of altitudinal adaptation of physiological and morphological traits of mainland populations (Pryor 1956; Williams and Ladiges 1985). In Tasmania, 37 provenances have been sampled across the geographical and ecological range of *E. pauciflora* (Fig. 1.2) with provenance differences supported by quantitative analysis of seedling traits in a glasshouse experiment (Gauli *et al.* 2015). Further investigation of the provenance structure of *E. pauciflora* found a strong association of provenance home-site altitude and climate differences in many quantitative traits. For example, lignotuber size decreased with increasing provenance home-site altitude, while increasing with maximum temperature of the warmest month (Gauli *et al.* 2015). Additionally, quantitative inbreeding coefficients (Q_{st}) were high for many traits in the study. Q_{st} values are estimates of the degree of population structure of a trait and are often used to examine whether that trait displays a signal of diversifying selection (Dutkowski and Potts 2012; Edelaar and Björklund 2011; Hamilton *et al.* 2013; Whitlock 2008). Significant trends with altitude and climatic variables combined with high Q_{st} values provides evidence of adaptive variation in many phenotypic traits within the species (Gauli *et al.* 2015). The association of many traits with altitude and climate variables indicates that genetic-based provenance variation within the species is largely driven by adaptation to the local environment. The ability of this species to grow environments has made it a target for restoration programs in Tasmania in drought prone environments with areas of large-scale tree decline (Bailey 2013). Little is known of the potential extended consequences of provenance translocations to the biotic community in these large-scale restoration plantings. Given the evidence of adaptive variation within *E. pauciflora*, it is reasonable to assume that such adaptation may extend to the biotic community. In wild mainland populations of *E. pauciflora*, an increase of leaf loss associated with insect herbivores and fungal pathogens with increasing altitude has been reported (Burdon and Chilvers 1974). Investigation of the potential extended effects of

adaptive variation in the species is important for our understanding of the possible biotic impact of provenance translocations in these restoration plantings. Such translocations could shift the ecological and evolutionary trajectory of the biotic community in these areas with a potential loss of, or unappreciated impacts on, biodiversity values of restored and adjacent native forests.

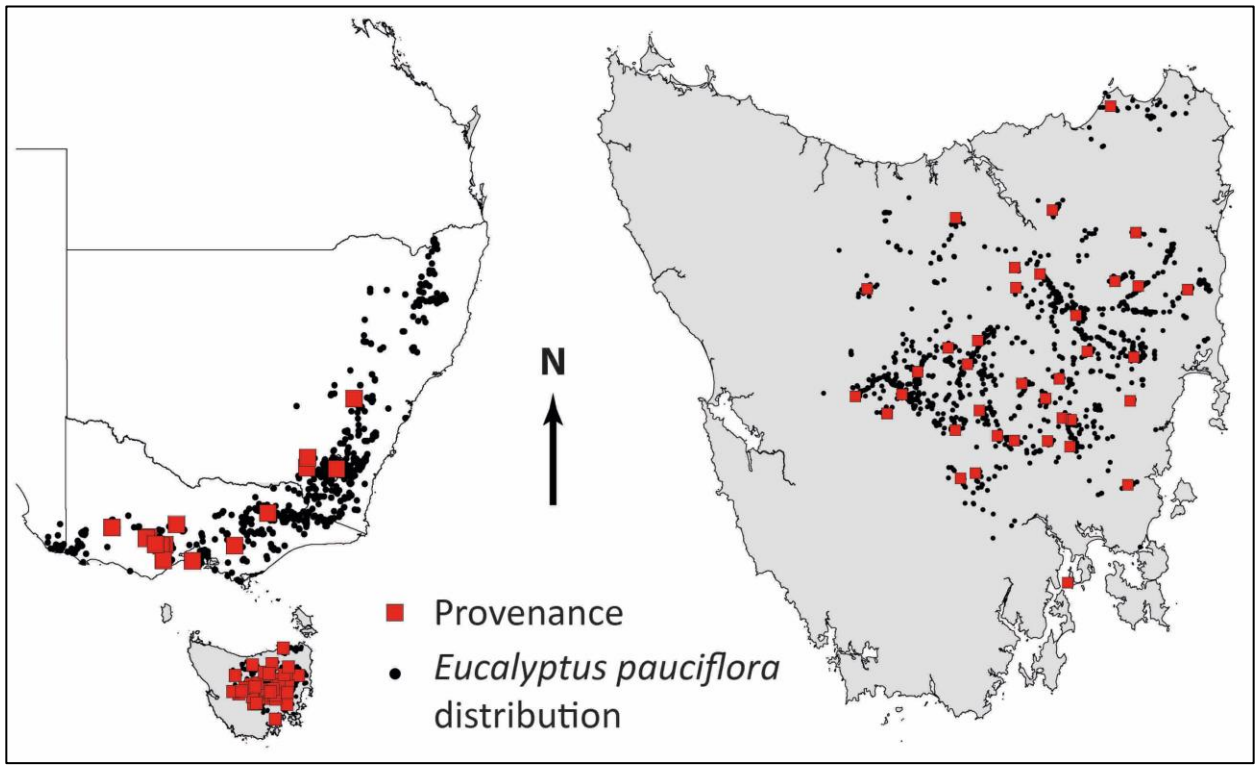

Fig. 1.2. Geographic distribution of *Eucalyptus pauciflora* and provenance locations in Australia (left) with Tasmania enlarged (right). Black dots show the reported records of species from Atlas of Living Australia (http://www.ala.org.au/) and Nature Values Atlas (https://www.naturalvaluesatlas.tas.gov.au/). Red squares show the location of provenances from Tasmania (37 provenances) and mainland Australia (13 provenances) included in field trials studied in this thesis. Figure provided by Peter Harrison.

1.5.3 Eucalyptus globulus

Eucalyptus globulus, commonly known as the blue gum, is native to Tasmania and south-eastern Australia and represent a species of commercial value in this thesis. The species is part of the subgenus *Symphyomyrtus* and is one of the most widely planted commercial forestry species in the world due to its rapid growth (Hillis 1984; Potts *et al.* 2004). *E. globulus* plantations are primarily used for paper pulp wood production (Hillis 1984; Potts *et al.* 2004), as well as being one of the main sources of eucalyptus oil (Boland *et al.* 1991). Trees are heteroblastic and occur naturally in protected valleys on light to medium clay loam as well as near coastal areas on sandy loam and range in size from a dwarfed coastal form to up to 70m (Boland *et al.* 2006). *E. globulus* is highly diverse across is geographic range with significant genetic-based provenance variation in morphological, growth and chemical traits (Dutkowski and Potts 1999; O'Reilly-Wapstra *et al.* 2013b). Due to its commercial importance, genetic variation within the species has been studied extensively.

Genetic variation within *E. globulus* was initially summarized by classifying the gene pool into 13 geographic races and 20 sub-races (Fig. 1.3) based on quantitative genetic variation in morphological and growth traits (Dutkowski and Potts 1999). Later assessment of neutral molecular markers showed that these races could be classified into three main geographic groupings - mainland Australia, western Tasmanian (which includes the King Island race in Bass Strait), and eastern Tasmania (which also includes the Furneaux island races) (Steane *et al.* 2006). Consistent with barriers to gene-flow, the main divergence is between mainland Australian and Tasmanian races (Jones *et al.* 2013). This latitudinal differentiation from mainland to Tasmanian races has also been shown in many quantitative traits, including wood properties (Stackpole *et al.* 2011) and foliar chemistry (O'Reilly-Wapstra *et al.* 2013b; Wallis *et al.* 2011). QTL studies have shown that many of the quantitative traits reporting significant differences between races in *E. globulus* are under genetic control, including detection of QTL for growth and wood properties (Freeman *et al.* 2009) and foliar chemistry (O'Reilly-Wapstra *et al.* 2011).

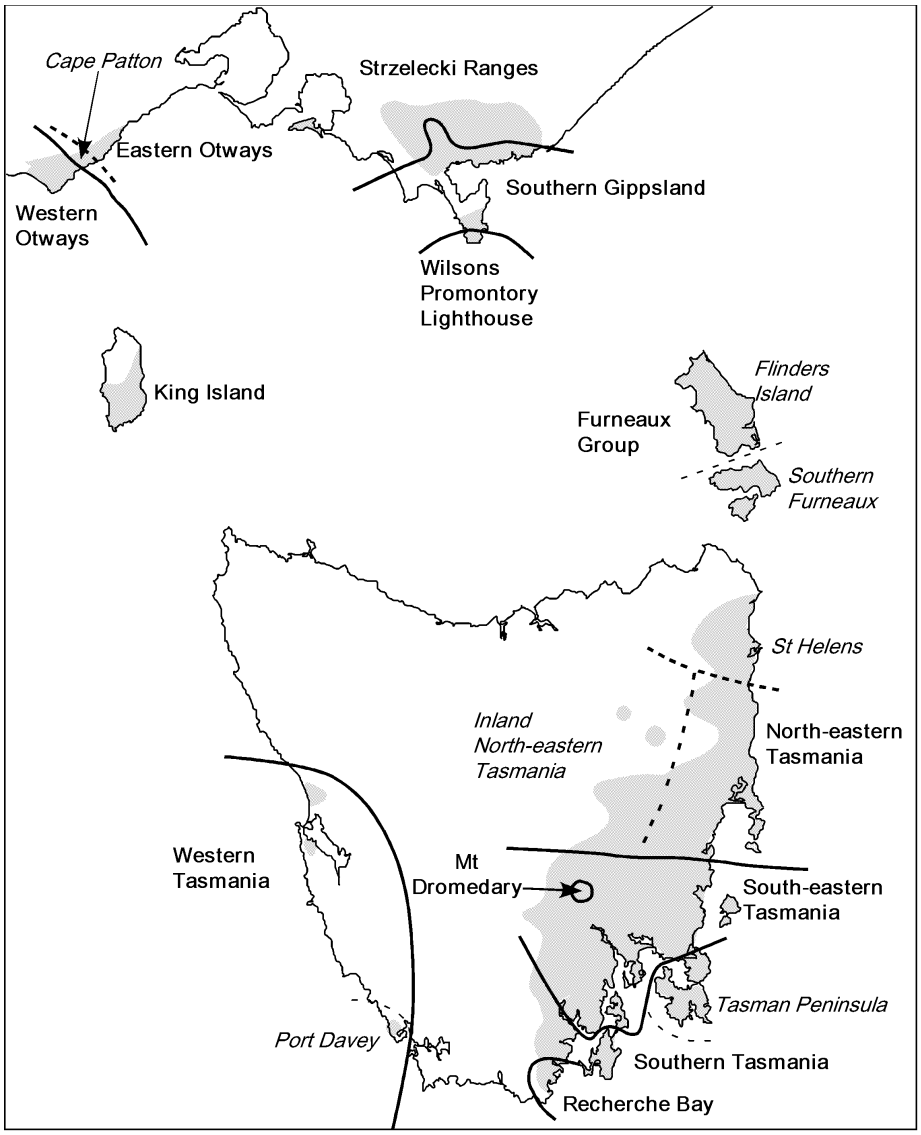

Fig. 1.3. Geographic distribution of *E. globulus* (shaded) and the delimitation races and sub-races as defined by Dutkowski and Potts (1999) (and slightly modified by Potts *et al.* 2004) of *Eucalyptus globulus*. Represented provenances from 13 sub-races were included in the present study. Figure is taken from (Potts *et al.* 2004)

The influence of genetic variation in *E. globulus* has been shown to extend to individual organisms and community-level responses. For example, significant variation between races of *E. globulus* has been reported for their susceptibility to fungal pathogens (Carnegie *et al.* 1994; Hamilton *et al.* 2013) and major pest insect (Jordan *et al.* 2002) and marsupial (O'Reilly-Wapstra *et al.* 2002) herbivores, as well as in the overall composition of the dependent canopy (Barbour *et al.* 2009c),

and under-bark (Barbour *et al.* 2009b) communities. Additionally, potential bioactive QTL in *E. globulus* have been detected with identification of QTL for fungal pathogen resistance in an F_2 mapping family (Freeman *et al.* 2008b) with the same family showing significant genetic variation in the resistance to defoliation by the autumn gum moth *Mnsamplae privata* (Jones et al. 2002). While extended genetic effects on the dependent canopy community have been documented, there is still much to determine. To progress the field of community genetics in forest trees with established extended genetic effects, such as *E, globulus*, the relative influence of these effects (Tack and Roslin 2011), the potential underlying mechanisms driving community differences (Hersch-Green *et al.* 2011), and the continuation of the field into a genomics perspective (Whitham *et al.* 2008) is needed.

1.6 Thesis structure and aims

This thesis uses the above three species from the genus *Eucalyptus* to examine the extended genetic effects on the canopy communities of forest tree species. The species encompass different subgenera and distribution patterns, as well as different conservation and economic significance. Chapter 1 is the general introduction, addressing the broad themes and background information for the thesis. Chapters 2 through to 6 are experimental chapters (Table 1.1) written in the format for publication as separate papers. Therefore, there may be some repetition in the thesis, particularly in the introduction sections of each chapter. Chapter 7 is the general discussion for the thesis, addressing the key findings and implications of the work presented in the experimental chapters.

The overall aim of the research presented in this thesis was to determine the relative importance of provenance effects on the dependent arthropod and fungal community, while exploring potential mechanisms driving genetic-based variation in community responses and identifying underlying QTL. Specific aims were to:

1) determine the relative importance of extended genetic effects in eucalypts in comparison to known factors influencing biotic communities, including environmental, yearly and ontogenetic effects.

2) examine the stability of the extended genetic effects in eucalypts across environments and years to understand the potential extended consequences of translocation of non-native genotypes in commercial, conservation and restoration practices.
3) identify and determine the relative importance of foliar chemical mechanisms driving genetic-based variation in dependent community responses in *E. globulus.*
4) provide insight into the regions of the *E. globulus* genome impacting on dependent community responses and determine if they coincide with those for potential foliar chemicals which may be potential mechanisms.

In chapter 2, the relative magnitude and stability of genetic-based effects on the dependent arthropod and fungal communities in eucalypts across sites and years was examined using *E. globulus* and *E. pauciflora*. Dependent communities for both eucalypt systems were assessed across two common garden trials to determine the relative magnitude of genetic-effects compared to site effects in eucalypts. Community assessment was repeated a second year in *E. pauciflora* allowing for comparison of genetic versus year effects. This chapter addresses the possible biotic consequences to provenance translocations and whether they are predictable across environments and over time.

In chapter 3, the relative magnitude of genetic and ontogenetic effects on dependent arthropod and fungal communities was examined and compared in the endangered heteroblastic tree *E. morrisbyi*. Additionally, potential underlying physiochemical mechanisms driving genetic-based community variation and the possible indirect genetic effect of a mammalian herbivore were investigated. This chapter provides a basis for further investigation of phytochemistry in subsequent chapters, as well as addressing the potential biotic consequences to management strategies of endangered species.

In chapter 4, the genetic basis of variation in cuticular wax compounds was investigated in *E. globulus* using quantitative genetic and QTL analyses. This chapter provides a basis for further investigation of the extended genetic effects of cuticular wax compounds in subsequent chapters, as well as insight into the genetic control of cuticular wax compounds in *E. globulus*.

In chapter 5, the potential chemical drivers of genetic-based provenance variation in the dependent foliar arthropod and fungal community of *E. globulus* was examined, focusing on the role of cuticular waxes and foliar terpenes. This chapter addresses the potential extended effects of adaptive variation to community-level responses in eucalypts.

In chapter 6, the underlying regions of the genome associated with the dependent arthropod and fungal community was investigated in *E. globulus*. Quantitative trait loci (QTL) analysis was undertaken and identified QTL for community traits were compared to previously reported QTL for foliar chemistry as well as the identified QTL for cuticular waxes in chapter 4. This chapter addresses whether underlying regions of the genome influencing individual community members are the same as those influencing community-level traits. Additionally, it assesses whether foliar chemistry influencing community-level responses at broader genetic-scales (chapter 5) underlie community-level QTL.

Table 1.1 Summary table of trial and assessment information for experimental chapters.

	Eucalypt species	Trial(s) assessed	Trial type	Assessment taken
Chapter 2	*E. globulus*	Salmon River	Provenance	Arthropod and fungal symptoms
		Temma	Provenance	Arthropod and fungal symptoms
	E. pauciflora	Bignals	Provenance	Arthropod and fungal symptoms
		Dungrove	Provenance	Arthropod and fungal symptoms
Chapter 3	*E. morrisbyi*	Oigles Rd.	Provenance	Arthropod and fungal symptoms
				Near-infrared spectroscopy (NIR)
				Possum browsing[2]
Chapter 4	*E. globulus*	Salmon River	Provenance	Foliar wax chemistry
		Woolnorth	QTL	Foliar wax chemistry
Chapter 5	*E. globulus*	Salmon River	Provenance	Arthropod and fungal symptoms[3]
				Foliar wax and terpene chemistry[4]
Chapter 6	*E. globulus*	Woolnorth	QTL	Arthropod and fungal symptoms
				Foliar wax, terpene and FPC chemistry[5]

[1] Both *E. pauciflora* trials were assessed over two years. All other trials underwent a single assessment.
[2] Possum browsing scores were previously assessed scores from the same trial at an earlier data originally published in Mann *et al.* (2012).
[3] The arthropod and fungal symptom data in chapter 5 is a subset of the data from the Salmon River trial in chapter 2 to match the trees assessed at the same time for foliar chemistry.
[4] The wax chemistry data in chapter 5 is the same as that from the Salmon River trial in chapter 4.
[5] The wax chemistry data in chapter 6 is the same data as that from the Woolnorth trial in chapter 4. The terpene chemistry data was originally published in O'Reilly-Wapstra *et al.* (2011) and the FPC chemistry data was originally published in Freeman *et al.* (2008a).

Chapter 2: Importance of community genetic effects across space and time: Insights from *Eucalyptus* provenance translocations

Abstract

Intra-specific genetic variation in forest trees can influence dependent communities and ecosystem processes, however, the significance of such extended genetic effects depends on their relative importance and stability across environments and through time. This is particularly true in regards to commercial, conservation and restoration practices as provenance translocations are increasingly being promoted as a response to climate change. We investigated the relative importance and stability of these extended genetic effects in the tree genus *Eucalyptus* using two species – *E. globulus* and *E. pauciflora*. In the case of *E. globulus*, the dependent arthropod and fungal canopy communities were quantified based on the abundance of 49 symptoms from 722 progeny from 13 geographic sub-races across two common garden trials. For *E. pauciflora,* 6 symptoms were quantified over two years from 238 progeny from 16 provenances across two common garden trials. Genetic main effects significantly influenced dependent canopy communities in both *E, globulus* and *E. pauciflora*, accounting for an average of 5.6% of the variation in community trait across sites and 2.8% across year and sites, respectively. However, site and year effects far outweighed those of genetic effects with site differences explaining approximately 3 times the variation in community traits in *E. globulus* and site and year differences both explaining approximately 6 times the variation in *E. pauciflora*. While the interaction terms involving genetic effects were significant in some community traits, broad trends in community effects associated with variation in home-site latitude (mainland vs Tasmanian sub-races) in the case of *E. globulus* and home-site altitude in the case of *E. pauciflora* were evident. These broad-scale trends were consistent with reported patterns of adaptive differentiation within each species, suggesting that there may be extended consequences of local adaptation. While small in comparison to site and year, the stability of such genetic effects highlights the importance of provenance choice in commercial, conservation and restoration practices as adaptive divergence among provenances within forest tree species, such as *Eucalyptus*, may have significant extended effects on biotic communities and ecosystem processes.

2.1 Introduction

Plant genetic variation has been shown to have extended genetic effects, influencing dependent communities and ecosystem processes in numerous plant systems (Hersch-Green *et al.* 2011; Whitham *et al.* 2006; Whitham *et al.* 2012). Foundation species, such as the forest trees of the genera *Populus*, *Quercus* and *Eucalyptus*, have been the model for community genetics studies due to their disproportionate influence on the environment (Whitham *et al.* 2010). Trees determine forest structure, creating locally stable environments, allowing for the development of associated communities and ecosystem processes (Ellison *et al.* 2005; Whitham *et al.* 2010). In forest trees, extended genetic effects have been shown to influence dependent communities, both above- (Busby *et al.* 2015; Lau *et al.* 2016) and below- (Gehring *et al.* 2014; Lamit *et al.* 2015) ground, as well as extending to higher level trophic interactions (Smith *et al.* 2011). These extended genetic effects can impact multiple facets of the community, from community member abundance, biodiversity and interactions to overall composition (Crutsinger *et al.* 2006; Gosney *et al.* 2014; Maddox and Root 1987). The influence of intra-specific genetic variation in forest trees on dependent communities have been observed at various genetic scales, including between clones (Fritz and Price 1988; Korkama *et al.* 2006; Robinson *et al.* 2012; Silfver *et al.* 2014; Whitham *et al.* 2006), families (Axelsson *et al.* 2015; Roche and Fritz 1997) and provenance (Barbour *et al.* 2009c; Gosney *et al.* 2014; Sinclair *et al.* 2015); in addition to the effects of interspecific hybridization (Bangert *et al.* 2006a; Dungey *et al.* 2000; Wimp *et al.* 2005).

Exploring how plant genetic variation impacts the surrounding communities and ecosystem is important for our understanding of the evolutionary processes that shape biological interactions (Whitham *et al.* 2012). It is also important for understanding the consequences of anthropogenic translocations. In the case of forest trees, this is especially important as provenance translocations are being increasingly promoted as a response to global climate change for the purpose of forestry (Gray *et al.* 2016), conservation (Aitken and Whitlock 2013) and restoration (Breed *et al.* 2013; Prober *et al.* 2016). Such assisted migration may occur both within (assisted gene flow) or beyond (assisted colonization) the current distribution of a species (Aitken and Whitlock 2013). For example, in the case of restoration a ‘local is best’ approach has long been thought to be optimal, but under climate change predictions non-local genotypes may be more suitable for targeted sites (Harrison *et al.* 2017; Jones 2013; Prober *et al.* 2016; Thomas 2011; Weeks *et al.* 2011). However,

the broader consequences of the translocation of genotypes across genetic and environmental barriers are yet unclear, as extended genetic effect may have on- and off-site impacts on dependent communities and ecosystem processes (Bucharova 2016; Frascaria-Lacoste and Fernandez-Manjarres 2012). Additionally, local adaptation of genotypes may provide resistance to major pests, which could be absent in non-local genotypes (Endler *et al.* 2010). While extended genetic effects have been established in many species, the evolutionary and ecological importance of these effects depends on their relative magnitude and stability across space and time (Hersch-Green *et al.* 2011; Keith *et al.* 2010).

Few studies have compared the relative magnitude and stability of genetic-based effects of forest trees on the dependent biotic community across sites and years. Instability was highlighted in an early study of intra-seasonal variation in arthropod communities on hybrid poplars grown in a common garden, where not only did the effect of plant genetics change through the season, but so did the relationship of the dependent community with phytochemistry (Wimp *et al.* 2007). Subsequent studies suggest that in general, environmental effects (site differences) appear to outweigh those of genetic effects, accounting for up to 4 times the variation in communities (Busby *et al.* 2014; Silfver *et al.* 2014; Tack and Roslin 2011). This appears to be the opposite for yearly differences, with genetic effects having a greater influence and stability through time (Busby *et al.* 2014; Keith *et al.* 2010). However, these studies still showed significant genetic effects across sites and years, regardless of the size of the effect. In focal tree species with underlying spatial and adaptive trends in inter-population genetic variation, the stability of these effects through space and time can provide insight into their evolutionary and ecological history and allow a better understand of the consequences of the translocation of non-local genotypes.

In the genus *Eucalyptus,* common garden experiments have shown intra-specific genetic variation influences dependent communities in two systems, the commercially important *Eucalyptus globulus* and the endangered *E. morrisbyi*. In *E. globulus,* extended genetic effects at the population-level have been shown to influence the canopy (Chapter 5; Barbour *et al.* 2009c), bark (Barbour *et al.* 2009b) and litter (Barbour *et al.* 2009a) communities, with effects on the canopy community also reported in *E. morrisbyi* (Gosney *et al.* 2014). In addition, two different common garden studies of inter-population effects on the canopy community of *E. globulus* appear to show

similar patterns, with underlying latitudinal trends in inter-population variation reflected in the dependent community composition (Chapter 5; Barbour *et al.* 2009c). This consistency between the studies hints at a possible stable extended genetic effect within the system, however, this has yet to be formally examined in eucalypts and little is known about the magnitude of these effects relative to non-genetic factors that are well-known to influence dependent communities, such as site and season. In the present study, two important eucalypt systems, *E. globulus* and *E. pauciflora*, are used to address the relative magnitude and stability of extended genetic effects on the dependent arthropod and fungal community through space and time. *Eucalyptus globulus,* is a commercially important forest tree native to the island of Tasmania and coastal regions of south-eastern Australia, but grown globally primarily for pulpwood production (Potts *et al.* 2004). It is one of the most genetically well-studied eucalypts, mainly planted outside its natural range, even within Australia (Costa e Silva *et al.* 2006), and here reflects an example of assisted colonization. *Eucalyptus pauciflora* is a species of restoration importance in Australia (Bailey 2013). It occurs naturally in Tasmania and south-eastern Australia and has one of the widest ranges of any eucalypt, from almost sea level to nearly 2000m (Boland *et al.* 2006; Williams and Potts 1996). In the present study, this species is used as an example consistent with assisted gene flow, as many restoration plantings in Australia are within the native range and the species is being used to test climate-adjusted provenancing strategies (Harrison *et al.* 2017).

2.2 Materials and Methods

2.2.1 Eucalyptus globulus

2.2.1.1 Field trials

The stability of genetic effects on the dependent arthropod and fungal community across space and time was examined using two common environment field trials for each *E. globulus* and *E. pauciflora* (Table 2.1). The two *E. globulus* trials were established in 2006 outside the natural range of *E. globulus*. The trials are located at Salmon River (41° 01’ S, 144° 52’ E; 110m above sea level) and Temma (41° 07’ S, 144° 45’ E; 120m above sea level) in northwest Tasmania, Australia, for breeding purposes. While only 40 km apart they occur on markedly different soil profiles and geology. The Salmon River trial is on red-brown clay on Cambrian inter-layered mudstone, siltstone and sandstone, while the Temma trial is on yellow-brown mottled clay on Precambrian mudstone. The trials were established using 140 open-pollinated families sampled

across the natural range of *E. globulus*, 140 of which were planted at the Salmon River trial and 124 at the Temma trial. These trees were grouped into 13 geographic sub-races (ie. native home-site provenances), representing the 13 geographic races of *E. globulus* following Dutkowski and Potts (1999). Family and sub-race identity were maintained in the trials. The trials were planted as randomized incomplete block designs with Salmon River consisting of 25 replicates and 13 incomplete blocks per replicate and Temma consisting of 25 replicates and 12 incomplete blocks per replicate. Families were planted as single-tree plots within replicates. Full trial details can be found in Hamilton *et al.* (2013). A total of 379 trees from Salmon River and 343 trees from Temma were sampled for the present study, originating from 13 sub-races. The population samples of each sub-race consisted of 5-13 families (128 families total) with 2-4 individuals per family (n = 722). 121 of the 128 sampled families were represented at both site locations.

2.2.1.2 Community assessment

Sampling for assessing the canopy foliar community on the 722 trees took place during March and April 2012. Twenty fully expanded adult leaves collected from each tree for assessment. Leaves were randomly sampled from a branch felled from the north side of each tree. The leaves were placed in paper envelopes and stored at 30°C to dry prior to assessment. The foliar arthropod (primarily herbivorous arthropods) and fungal community was assessed using a symptom-based approach through consultation with entomologists. While widely used in community genetics studies (Barbour *et al.* 2009c; DeWoody *et al.* 2013; Keith *et al.* 2010; Rönnberg-Wästljung *et al.* 2006; Tack and Roslin 2011), symptom-based data may be unrelated to the actual community parameters of interest, such as organism abundance and richness (Bito *et al.* 2011). However, there is correlated evidence in both the fossil record and living forest suggesting that symptom-based data can be used to interpret organism richness and composition (Carvalho *et al.* 2014). Scoring was done as the presence/absence of individual symptoms on each of the 20 leaves per tree. A total of 49 individual symptoms were identified across the two trials (See Appendix A Fig. 2.S1 for photographs of symptoms). 39% of symptoms were unique and assignable to individual taxa. The remaining included some symptoms which were likely different symptoms of the same or different life history stage of the same taxa (but maintained separate for analysis) and several symptoms represent multiple organism damage types where one type of damage was subsequent to another.

The community data for each *E. globulus* tree used for calculations and analyses was thus the number of leaves, out of the 20 collected per tree, affected by a given symptom.

Table 2.1 Summary table of experimental design and community assessment.

	E. globulus		*E. pauciflora*	
Trials assessed	Salmon River	Temma	Bignals	Dungrove
Years assessed	2012	2012	2014 & 2015	2014 & 2015
No. provenances/sub-races	13	13	16	16
No. families	128	121	239	239
No. replicates[1]	5	6	1	2
No. trees	379	343	90	148
No. of assessed symptoms	47	44	6	6
Type of symptom assessment	Presence/absence of a given symptom scored on 20 sampled leaves per tree (0,1)		2-minute, whole tree visual assessment of the estimated proportion of leaves affected by a given symptom (0.00-1.00)	

[1] The number of replicates for the *E. globulus* trials referrer to the spatial replicates assessed within the trial. Not all families were assessed in each replicate. The two replicates at Dungrove for *E. pauciflora* were treated as separate sites for the purpose of this study.

2.2.2 Eucalyptus pauciflora

2.2.2.1 Field trials

The two *E. pauciflora* trials sites were established in 2010 as part of a large-scale restoration planting at Dungrove (42° 16' S, 146° 53' E; 569m above sea level) and Grassy Hut (42° 23' S, 147° 04' E; 422m above sea level) in the Derwent Valley of Tasmania, Australia, which are located approximately 38 km apart. Trial site altitudes are in the mid-altitudinal range for Tasmania *E. pauciflora*. Both trials were planted on cleared agricultural land consisting of fine sandy loam soil on Permian mudstone with remnant patches of native eucalypts. The Dungrove trial site is surrounded by fragmented *E. pauciflora* and *E. tenuiramis* woodlands, while an adjacent *E. rubida, E. pauciflora,* and *E. tenuiramis* woodland is present at Grassy Hut. This study focuses on the genetic trials that have been embedded within the larger restoration plantings (Bailey 2013). Both trials were derived from the same open-pollinated seedlots, collected from 52 provenances of *E. pauciflora* sampled across the natural range of the species (37 Tasmanian, 15 mainland Australian). The Tasmanian provenances comprised 281 individual tree seedlots (families) with identity maintained, whereas the mainland provenances comprised bulk open-pollinated seedlots collected from between one to nine trees per provenance. The trials were planted using a randomized row-column design produced through CycDesigN 4.0 (CycSoftware 2009), with each

trial consisting of 8 replicates of 20x20 tree plantings. Each of the 281 Tasmanian families were represented once in each replicate, with the remaining 119 trees in each replicate consisting of the 15 mainland provenances represented up to nine times in each replicate.

In the present study, a total of 148 trees from two replicates (treated as independent sites for the purpose of this study) at Dungrove and 90 trees from one replicate at Grassy Hut were assessed for their dependent foliar arthropod and fungal community. Due to mortality and retention of juvenile foliage in many trees at both sites, this subset of 148 from the planted 281 families allowed all assessed families to be represented across both sites and the same individuals within each site to be sampled in both years. These trees were from 16 provenances (14 Tasmanian and 2 Mainland Australian) with 2-9 families per provenance and one tree per family (total n=239; See Appendix A Table 2.S1 for provenance details). All families were represented over both years at each of sites. The two replicates at Dungrove (Rep 1 and Rep 8) are located over 500m apart and separated by a remnant patch of *E. pauciflora* and *E. tenuiramis*, with Rep 1 planted 30m of the remnant patch and Rep 8 planted on the edge of the trial over 150m from the remnant patch.

2.2.2.2 Community assessment

Assessment took place during March 2014 and was repeated during the same period in 2015, to further examine the stability of community genetic effects over time. The foliar arthropod (primarily herbivorous arthropods) and fungal community was assessed using a symptom-based approach of preselected damage types. In total, 6 symptoms were selected based on their dominance and/or ease of identification in the field, and comprised of 5 arthropod symptoms and 1 fungal symptom. Damage types included leaf "scalloping" largely performed by species of the chysomelid leaf beetle genus *Parospisterna*, leaf "notching" performed by *Paropsisterna* larvae, leaf "smoothing" performed by Lepidoptera larvae, fungal pathogen damage primarily associated with the genus *Teratosphaeria*, leaf tip damage caused by *Doritifera oxleyi* moth larvae, and leach chewing associated with the leaf beetle genus *Cadmus* (See Appendix A Fig. 2.S2 for photographs of symptoms). The abundance of each symptom on each tree was quantified using estimates of the proportion of fully expanded adult foliage from the assessed years' growth which had been damaged. These estimates were based on two-minute visual scans of the canopy of each tree which, due to the small tree size (less than 3 m on average), was possible from the ground.

2.2.3 Statistical analysis

Compositional variation in the arthropod and fungal community for both *E. globulus* and *E. pauciflora* systems was analyzed at the multivariate level using Permanova+ in Primer 6 (version 6.1.3; Roborough, Plymouth, UK). Compositional variation among samples was summarized using a Bray-Curtis dissimilarity matrix in Primer 6. Individual symptom data were standardized by unit maxima for calculation of the Bray-Curtis dissimilarities. For each system, Permanova+ analysis of the Bray-Curtis dissimilarity matrix fitted models which included genetic factors and experimental design features as follows:

E. globulus

$$y = \mu + \text{Site} + \textit{Rep(Site)} + \text{Sub-race} + \textit{Family(Sub-race)} + \text{Site*Sub-race} + \textit{Site*Family(Sub-race)} + \text{residuals}$$

E. pauciflora

$$y = \mu + \text{Year} + \text{Site} + \text{Provenance} + \text{Year*Site} + \text{Year*Provenance} + \text{Site*Provenance} + \text{residuals}$$

where Site, Sub-race and their interaction are fixed effects and *Rep(Site)* and *Family(Sub-race)* are random effects in the *E. globulus* system and all factors are fixed in the *E. pauciflora* system. In both systems, the relative influence of each factor on the compositional dissimilarity was calculated from the variance components obtained from the Permanova+ analyses. All Permanova+ analyses were done using type III sums of squares.

To summarize the patterns of sub-race or provenance variation in the dependent community composition, canonical analyses of principal coordinates (CAP) were undertaken using the Bray-Curtis dissimilarity matrices for both the *E. globulus* and *E. pauciflora* systems using *capscale* from the package *vegan* in R (R Core Team 2013). In contrast with unconstrained ordination analyses, such as multi-dimensional scaling (MDS), CAP analyses may uncover otherwise masked patterns of variation using constrained ordination methods maximizing designated group differences (Anderson and Willis 2003). Groups for the *E. globulus* CAP analysis were designated

as sub-race within site. Groups for the *E. pauciflora* CAP analysis were designated as provenance within site within year. Differences between groups for both systems were summarized through ordination of centroids from the first two CAP axes. To examine the influence of independent symptoms on the community, group mean values for all organisms were fit as vectors into their respective canonical space with *envfit* from the package *vegan* in R. Only symptoms showing significance in the ordination space were plotted.

To further explore community genetic effects, mixed-model analyses using the models described above were undertaken using Bayesian inference on calculated univariate community parameters and independent symptom abundances with JAGS (Plummer 2003) in R using the package *R2jags*. For the *E. globulus* system, only organisms occurring on ten percent or more of the trees were analyzed at the univariate level. All six symptoms from the *E. pauciflora* were analyzed regardless of their proportion across the study. The community parameters calculated from the multivariate data set included total abundance, symptom richness, Shannon-Weiner diversity and Peilous' evenness, which were calculated using the function *diversity* from the package *vegan* in R. All community parameters for both study systems were analyzed assuming a Gaussian distribution. Due to the binary nature of the data and exploratory analysis indicating overdispersion, independent organisms from the *E. globulus* system were analyzed assuming a beta-binomial distribution. Independent organism abundances for *E. pauciflora* were analyzed assuming a beta distribution, due to the proportional nature of the data. All Bayesian analyses were run using half-Cauchy priors and uninformative initial values with three MCMC chains, a thinning of 1, a burnin of 10000, and 100000 iterations. Models followed those of the multivariate analyses for each system. Significance of individual effects in the model was determined through a drop-one approach in comparison of deviance information criterion values (DIC) from the Bayesian analysis output (Zuur *et al.* 2013). Significance of an individual effect was designated as anything greater than a 3-point increase in the DIC (ΔDIC) with the removal of the effect of interest (Spiegelhalter *et al.* 2002). Effect sizes for community parameter and individual symptoms were calculated from the sums of squares calculated during the univariate analyses (see footnote of Table 2.2).

Underlying patterns of community variation in the two systems were explored using Bayesian linear regressions of the respective sub-race and provenance arithmetic means across sites and

years. The response variables were the first CAP axis, total abundance, symptom richness and a single independent symptom of interest. The symptoms fitted were the ones showing significant genetic differences among su-races/provenances and the most significant variation with latitude for *E. globulus* and altitude for *E. pauciflora*. CAP analyses were redone for the regression analyses, maximizing differences between sub-races in *E. globulus* and provenances in *E. pauciflora* alone in the same manner as previously described. Regressions were performed using JAGS through the package *R2jags* in R under a normal distribution, running 3 MCMC chains, a thinning of 1, a burnin of 10000, and 100000 iterations. Initial values were set as the results from a simple linear regression using *lm* from the base *stats* package in R. R^2 values for the regressions were calculated from the results of a Bayesian equivalent of a Pearson correlation performed using the R package *R2jags* and running 3 MCMC chains, a thinning of 1, a burnin of 10000, and 100000 iterations. Initial values were set as the results from a simple Pearson correlation of the predicted values from the regression analyses with the original values using *cor* from the base *stats* package in R. Significant regressions were designated as those with a posterior probability greater than 0.95 that the independent variable increased/decreased with the dependent variable, but with a Bonferroni adjustment for multiple testing (n=4) applied resulting in a threshold probability of 0.9875.

2.3 Results

2.3.1 Eucalyptus globulus

In *E. globulus*, Permanova+ showed significant variation in the dependent arthropod and fungal community composition between both sub-races (Pseudo-$F_{12,721}$=2.6, p<0.0001) and family within sub-races (Pseudo-$F_{115,721}$=1.2, p<0.0001), with these genetic main effects accounting for 5.6% of the total variation among trees (Table 2.2). Site differences were also significant (Pseudo-$F_{1,721}$=26.9, p<0.0001) and their influence was far greater than that of the genetic-based main effects, accounting for 18.8% of the total variation. In comparison, the significant random replicate within site effect (Pseudo-$F_{9,721}$=2.0, p<0.0001) was relatively minimal, accounting for only 1.3% of the total variation. A significant interaction effect between sites and sub-races was detected (Pseudo-$F_{12,721}$=1.5, p=0.0007), indicating the sub-race effect in *E. globulus* on dependent community composition is not completely stable across environments. However, this effect only accounted for 1.4% of the total variation, and the magnitude of the interaction was less than half

of that of the genetic main effects. The significant main effect of sub-race was due to a consistent difference in a component of the canopy community (CAP1) between mainland and Tasmanian sub-races and the interaction appeared to be due mainly to site-specific differences in the affinities of the communities developed on the canopies of trees from the Bass Strait island sub-races (Fig. 2.1). The random site by family within sub-race interaction effect was not significant. In total, genetic and spatial main effects and interactions accounted for 27.2% of the variation in dependent arthropod and fungal community composition.

All calculated community parameters showed significant differences between sub-races of *E. globulus* (avg. ΔDIC=9), accounting for an average of 2.8% of the variation in these parameter. Across community parameters, *E. globulus* showed little environmental influence with only abundance showing significant differences between sites (ΔDIC=65; Table 2.2). This was due a greater symptom abundance observed in trees from Salmon River (mean 74±0.7) than Temma (mean 65±0.9). The random replicate within site and family within sub-race main effects were not significant for any of the calculated community parameters. Additionally, neither of the genetic by site interaction effects were significant for any of the community parameter, indicating that genetic sub-race effects on community parameters are relatively stable across environments.

Table 2.2. Grand means[1], standard deviation (SD) and summary of effect sizes for *E. globulus*. Shown are the effect sizes[2] and significance (bold are significant)[3] of the fixed site and sub-race (Sub) main effects, the random replicate within site (*Rep*) and family within sub-race (*Fam*) main effects, the fixed interaction effect of site and sub-race (SxS) and the random interaction effect of site and family within sub-race (*SxF*) from multivariate analyses of community composition (Permanova+) and univariate analyses of community parameters and assessed symptoms.

	MEAN	SD	Site	*Rep*	Sub	*Fam*	SxS	*SxF*	Resid
Permanova+									
Community Composition	-	-	**0.19**	**0.01**	**0.03**	**0.03**	**0.01**	0.00	0.73
Community Parameters									
Abundance[4]	69.8	15.8	**0.09**	0.02	**0.03**	0.01	0.02	0.01	0.82
Richness	10.7	2.4	0.00	0.01	**0.02**	0.01	0.00	0.02	0.94
Shannon	1.9	0.2	0.00	0.02	**0.04**	0.01	0.00	0.02	0.92
Evenness	0.8	0.0	0.00	0.01	**0.02**	0.02	0.00	0.02	0.93
MEAN	-	-	*0.02*	*0.01*	*0.03*	*0.01*	*0.00*	*0.02*	*0.90*
Symptoms									
Paropsisterna spp.	16.3	3.3	**0.20**	**0.02**	**0.03**	0.03	0.02	0.01	0.68
Aulographina eucalypti 1	13.2	5.9	**0.05**	0.00	**0.03**	0.02	0.01	0.01	0.87
Teratosphaera sp. 1	10.0	4.3	0.00	0.01	**0.12**	0.07	0.01	0.01	0.78
Plesanemma fucata	6.3	3.8	**0.17**	0.01	**0.03**	0.03	**0.02**	0.01	0.74
Teratosphaera sp. 2	5.2	4.6	**0.27**	**0.02**	**0.03**	0.02	0.00	0.03	0.63
Gonipterus sp. complex	4.6	2.6	0.00	0.00	0.00	0.02	0.02	0.02	0.93
Aulographina eucalypti 2	2.5	3.3	**0.10**	0.00	0.01	0.02	0.01	0.02	0.84
Cadmus excrementarius 1	1.9	2.5	0.00	0.00	**0.06**	0.05	0.00	0.01	0.88
Lepidoptera: *Psychidae*	0.9	2.6	**0.11**	0.00	0.03	0.06	0.00	0.02	0.78
Cadmus excrementarius 2	0.8	1.2	**0.02**	0.00	**0.05**	0.05	0.00	**0.01**	0.87
Pseudocercospora sp. 1	0.5	1.6	**0.03**	0.00	0.00	0.06	0.00	0.01	0.90
Coleoptera sp. 1	0.4	0.8	0.00	0.00	0.00	0.01	0.01	0.01	0.97
Pseudocercospora sp. 2	0.3	1.6	**0.00**	0.00	0.00	0.00	0.00	0.00	0.99
Teratosphaera sp. 4	0.3	1.0	0.00	0.00	0.00	0.03	0.03	0.08	0.87
Lepidoptera sp. 2	0.2	1.3	**0.01**	0.00	0.01	0.01	0.00	0.01	0.96
Teratosphaera sp. 5	0.2	1.3	0.00	0.00	0.01	0.08	0.09	**0.39**	0.43
Cadmus cognatus	0.2	0.9	0.00	0.00	0.00	0.00	0.00	0.01	0.99
Glycaspis cameloides (1st instar)	0.1	0.4	0.00	0.00	0.01	0.01	0.01	0.01	0.96
Lepidoptera: Leaf miner	0.1	0.3	**0.41**	**0.02**	**0.04**	0.01	0.00	0.02	0.50
MEAN	-	-	*0.07*	*0.00*	*0.03*	*0.03*	*0.01*	*0.03*	*0.82*

[1]Grand means for symptoms were calculated as the average number of leaves out of the 20 collected affected by a symptom on a tree.

[2]Effect sizes were calculated from the sum of squares (SS) from individual analyses as SS_{effect}/SS_{total}, expect for community composition which was calculated in the same manner from the variance components obtained from the Permanova+ analysis rather than the sum of squares.

[3]Boldface effect sizes indicate that the factor associated with the effect size (ie. Site, Rep, Sub-race, Family and interaction effects) were statistically significant based on Bayesian model comparison standards of a greater than 3-point increase in the deviance information criterion (DIC) from a hierarchical analysis excluding the factor of interest.

[4]Abundance is the sum of all assessed symptom grand means.

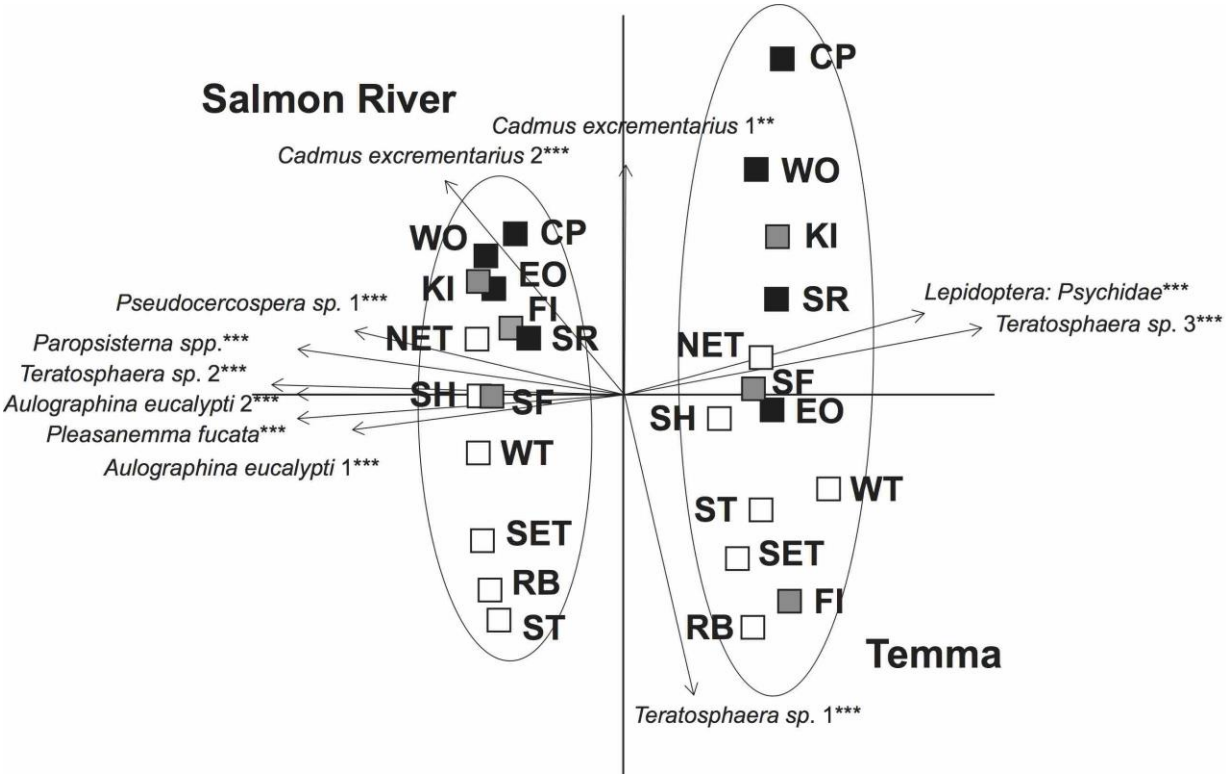

Fig. 2.1. Ordination of site (Salmon River and Temma) by *E. globulus* sub-race variation in dependent arthropod and fungal community composition using a canonical analysis of principal coordinates (CAP). The x axis is the first CAP axis (57.5% variance explained) and the y is the second CAP axis (9.1% variance explained). Black squares represent the mainland Australian sub-races (CP – Coastal Plain, EO – Eastern Otways, SR – Strzelecki Ranges, WO – Western Otways), grey squares the Bass Strait island sub-races (FI – Flinders Island, KI – King Island, SF – Southern Furneaux) and white squares the Tasmanian subraces (NET – North-eastern Tasmania, RB – Recherche Bay, SH – St. Helens, SET – South-eastern Tasmania, ST – Southern Tasmania, WT – Western Tasmania). Significant individual symptoms are displayed as vectors.

At the individual symptom level, sub-race differences significantly influenced 8 of the 19 analyzed symptoms (mean ΔDIC=24), accounting for an average of 4.9% of the variation per significant symptom (Table 2.2). Most notable of these was the fungal pathogen, *Teratosphaeria sp.*1, which displayed no significant site differences and a significant sub-race effect (ΔDIC=77) accounting for 11.8% of the variation among trees. Site differences had a substantial effect on most symptoms, significantly influencing 11 of the 19 analyzed symptoms in *E. globulus* (mean ΔDIC=94) and

accounting for an average of 13.7% of the variation per significant symptom (Table 2.2). The random replicate within site effect was significant for 3 of the 19 symptoms, showing a minor impact of within site spatial variation. The random family within sub-race effect showed no significant influence for any symptom. The interaction between site and sub-race was significant only for the abundance of the moth larvae, *Plesanemma fucata* (ΔDIC=77), while the random site by family within sub-race interaction effect was significant for the chrysomelid beetle, *Cadmus excrementrarius* 2 (ΔDIC=23), and the fungal pathogen, *Teratosphaeria sp.* 5 (ΔDIC=234; Table 2.2). Overall, most individual symptoms are influenced more by site than that of genetic effects, however, genetic effects are the primary determinant of some symptoms, and these appears to be relatively stable across sites.

As noted, the observed sub-race variation in dependent community composition for *E. globulus* at both sites is primarily due to differences between the mainland Australian and Tasmanian sub-races (Fig. 2.1). Vector fitting indicated that while most symptoms appear to be contributing to differences between sites, differentiation between the mainland Australian and Tasmanian sub-races appears to be due to differences in the abundance of *Teratosphaeria sp.* 1 and *Cadmus excrementarius* 1. These two symptoms showed the largest sub-race effect size from the univariate analyses, as well as indicating genetic-based stability across environments (Table 2.2). The differentiation in dependent communities between the mainland and the more southerly Tasmanian sub-races of *E. globulus* is further supported by significant regressions of sub-race mean score on the first CAP axis of an ordination maximizing sub-race differences with home-site latitude (Fig. 2.2). This latitudinal relationship was also detected in symptom richness and the frequency of *Teratosphaeria spp*., which increase (mainland > Tasmanian sub-races) and decrease (mainland < Tasmanian sub-races), respectively, with increasing latitude (Fig. 2.2).

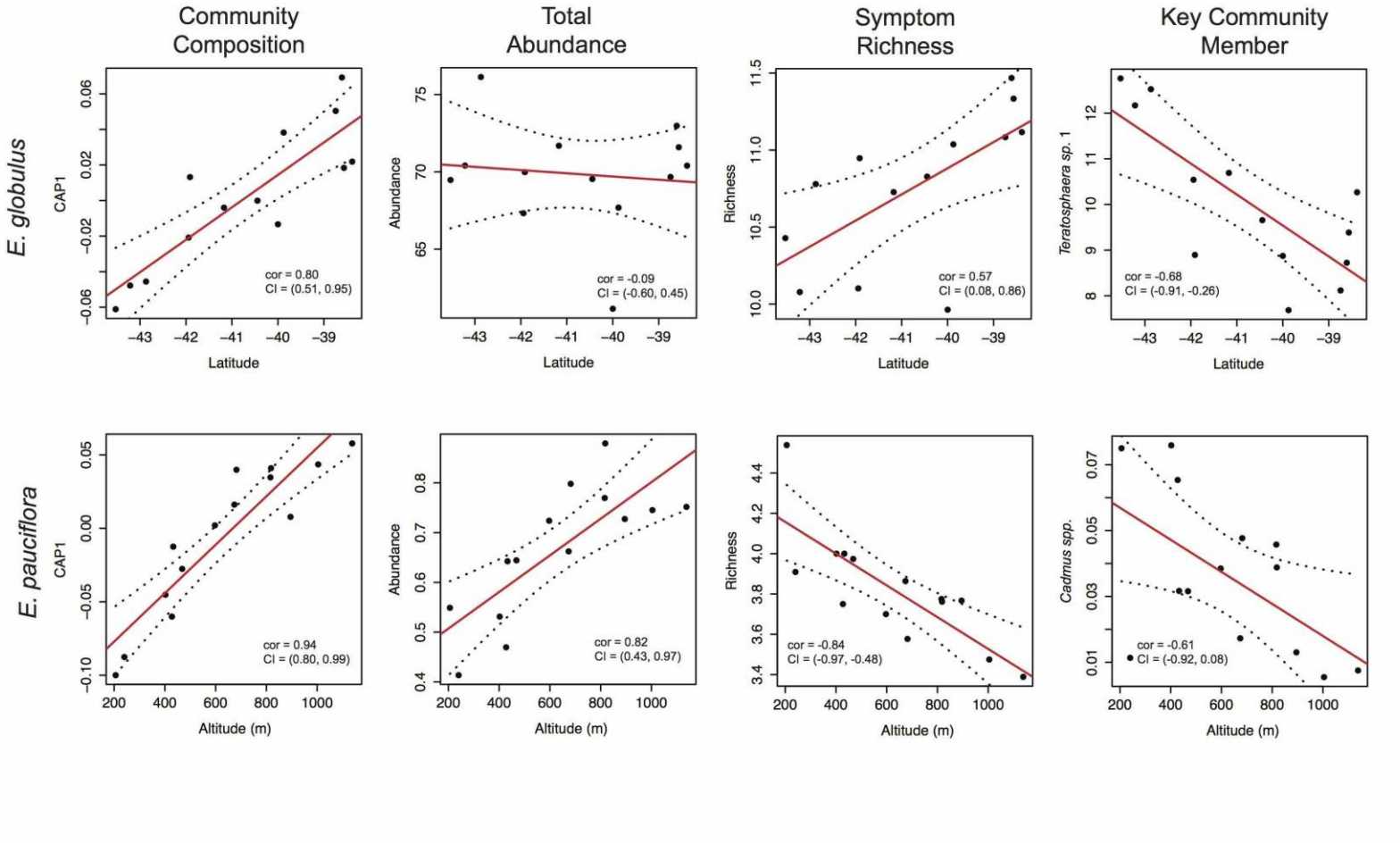

Fig. 2.2. Scatter plots showing the relationship between community variables and sub-race home site latitude (negative values are more southerly) in *E. globulus* and provenance home-site altitude (meters above sea level) in the Tasmanian *E. pauciflora*. *E.* globulus values are arithmetic sub-race means calculated across sites, while *E. pauciflora* provenance means are calculated across years and sites. The regression line (red line) and 95% confidence intervals of the linear relationship are presented, along with the Bayesian R^2 and 95% confidence intervals in the lower corners of each plot. Significant regressions with a posterior probability greater than 0.95 are in bold, with an asterisk indicating those still significant after a Bonferroni adjustment of the probability to greater than 0.9875. Community composition is represented by the first axis from the CAP analyses and key community members are the symptoms that showed significant differences among sub-races/provenances and the most significant relationship with latitude for *E. globulus* and altitude for *E. pauciflora*.

2.3.2 Eucalyptus pauciflora

In *E. pauciflora*, Permanova+ revealed significant variation in the dependent arthropod and fungal community composition among provenances (Pseudo-$F_{15,475}$=3.0, p<0.0001), with the genetic main effect accounting for 2.8% of the total variation in community composition among trees (Table 2.3). The effects of site (Pseudo-$F_{2,475}$=61.4, p<0.0001) and year (Pseudo-$F_{2,475}$=111.0, p<0.0001) were also significant and accounted for 15.7% and 19.0% of the total variation in dependent community composition among trees, respectively (Table 2.3). This was six times greater than the genetic main effect in *E. pauciflora*. All interaction terms between year, site and provenance effects were also significant in the Permanova+ analysis. Year by site (Pseudo-$F_{2,475}$=23.4, p<0.0001) accounted for 11.7%, year by provenance (Pseudo-$F_{15,475}$=1.6, p=0.0005) 1.8%, site by provenance (Pseudo-$F_{30,475}$=1.9, p<0.0001) 3.9% and year by site by provenance (Pseudo-$F_{30,475}$=1.4, p=0.0008) 3.7% of the variation among samples. Site and year effects are clearly the main source of the variation in dependent community composition in *E. pauciflora*, with genetic effect varying significantly with site and year. In total, main effects and interactions accounted for 58.8% of the variation in dependent arthropod and fungal community composition.

Of the calculated community parameters, total abundance was the only to show a significant provenance effect (ΔDIC=18), accounting for 4.8% of the total variation among trees (Table 2.3). Site differences in community parameters were far more influential showing a significant influence on all but symptom richness (mean ΔDIC=51), accounting for an average of 9.5% of the total variation per parameter (Table 2.3). Differences between years was only significant for Shannon-Weiner diversity (ΔDIC=31) and Pielous' evenness (ΔDIC=29), accounting for 6.5% and 6.2% of the total variation, respectively. The year by site interaction effect was significant for all community parameters (mean ΔDIC=58) accounting for an average of 10.3% of the variation among trees. None of the genetic by environment and/or time interaction effects were significant for the calculated parameters in *E. pauciflora*, indicating that the significant provenance effect on total abundance is relatively stable across sites and years despite its small effect in comparison to the site effect (Table 2.3).

Table 2.3. Grand means[1], standard deviation (SD) and summary of effect sizes for *E. pauciflora.* Shown are the effect sizes[2] and significance (bold are significant)[3] of the fixed main effects of year, site, and provenance (Prov), and the interaction effects of year and site (YxS), year and provenance (YxP), site and provenance (SxP), and year and site and provenance (YxSxP) from multivariate analyses of community composition (Permanova+) and univariate analyses of community parameters and assessed symptoms of *E. pauciflora*.

	MEAN	SD	Year	Site	Prov	YxS	YxP	SxP	YxSxP	Res
Permanova+										
Community Composition	-	-	**0.19**	**0.16**	**0.03**	**0.11**	**0.02**	**0.04**	**0.04**	0.41
Community Parameters										
Abundance[4]	0.67	0.40	0.00	**0.29**	**0.05**	**0.05**	0.00	0.02	0.00	0.58
Richness	3.8	1.2	0.00	0.00	0.02	**0.23**	0.01	0.03	0.01	0.70
Shannon	0.9	0.3	**0.06**	**0.03**	0.02	**0.10**	0.01	0.03	0.02	0.73
Evenness	0.6	0.2	**0.06**	**0.06**	0.01	**0.03**	0.01	0.02	0.00	0.82
MEAN	-	-	*0.03*	*0.10*	*0.02*	*0.10*	*0.00*	*0.02*	*0.01*	*0.71*
Symptoms										
Paropsisterna spp.	0.27	0.24	**0.11**	**0.27**	0.05	**0.04**	**0.01**	**0.04**	0.02	0.47
Paropsisterna spp. (larvae)	0.13	0.23	**0.10**	**0.17**	0.06	**0.15**	0.02	0.05	0.02	0.43
Teratosphaera spp.	0.08	0.15	**0.12**	**0.07**	0.04	**0.07**	0.01	0.00	0.00	0.68
Lepidoptera (larvae)	0.08	0.10	**0.15**	**0.01**	0.04	**0.03**	0.00	0.04	0.01	0.72
Doritifera oxleyi	0.05	0.07	**0.08**	**0.05**	0.00	**0.00**	0.01	0.05	0.01	0.80
Cadmus spp.	0.03	0.08	0.00	**0.09**	**0.07**	0.00	0.01	0.06	0.01	0.76
MEAN	-	-	*0.09*	*0.11*	*0.04*	*0.05*	*0.01*	*0.04*	*0.01*	*0.64*

[1]Grand means for individual symptoms were calculated as the average proportion of canopy damage per tree.

[2]Effect sizes were calculated from the sum of squares (SS) from individual analyses as SS_{effect}/SS_{total}, expect for community composition which was calculated in the same manner from the variance components obtained from the Permanova+ analysis rather than the sum of squares.

[3] Boldface effect sizes indicate that the factor associated with the effect size (ie. Year, Site, Provenance and interaction effects) were statistically significant based on Bayesian model comparison standards of a greater than 3-point increase in the deviance information criterion (DIC) from a hierarchical analysis excluding the factor of interest.

[4]Abundance is the sum of all assessed symptom grand means.

At the individual symptom level, provenance differences significantly influenced only the abundance of the chrysomelid beetle, *Cadmus spp.* (ΔDIC=10), accounting for 7.1% of the variation among trees (Table 2.3). Site differences significantly influenced all 6 assessed symptoms (mean ΔDIC=55) accounting for an average of 11.2% of the variation per symptom, while differences between years significantly influenced all but *Cadmus spp.* (mean ΔDIC=46), also accounting for an average of 11.2% of the variation per significant symptom. The year by site interaction effects was significant for all but *Cadmus spp.* (mean ΔDIC=36), accounting for an average of 11.2% of the variation per significant symptom, while the year by provenance and site

by provenance interaction effects were only significant for *Paropsisterna spp*. and the year by site by provenance interaction effect was not significant for any of the symptoms (Table 2.3). Overall, year and site effects are the primary determinant of symptom abundances, while the significant provenance effect on *Cadmus spp*. appears to be relatively stable across sites and years with an influence comparable to that of the significant site effect (Table 2.3).

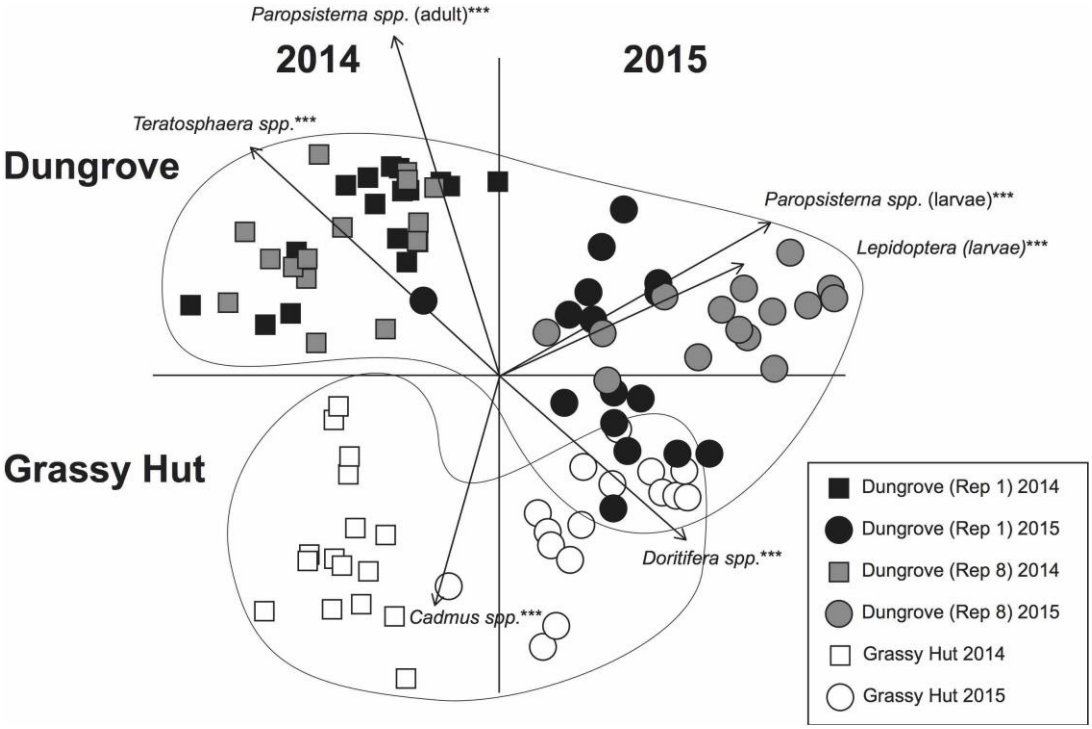

Fig. 2.3. Ordination of year, site, and provenance variation in dependent community composition in *E. pauciflora* using a canonical analysis of principal coordinates (CAP). The x axis is the first CAP axis (34.6% variance explained) and the y is the second CAP axis (28.8% variance explained). Squares represent provenances from the 2014 and circles the 2015 assessment. White squares and circles are provenances from the Grassy Hut site, black squares and circles from replicate 1 at the Dungrove site and grey squares and circles from replicate 8 at the Dungrove site. Symptoms are displayed as vectors.

The observed variation in the dependent canopy community of *E. pauciflora* is primarily due to the two assessed years and the two primary sites (Grassy Hut and Dungrove) with less differentiation between the two replicates within Dungrove (Fig. 2.3). Yearly difference appears to be driven by four of the six assessed symptoms, with a greater abundance of the fungal pathogen,

Teratosphaeria spp., in 2014 and a greater abundance of the arthropods *Paropsistera* spp. larvae, *Lepidoptera* larvae, and *Doratifera* spp. in 2015. Differences between the Grassy Hut and the Dungrove sites appear to be driven by adult *Paropsisterna* spp. and *Cadmus* spp., with a greater abundance of *Paropsisterna* spp. at Dungrove and a greater abundance of *Cadmus* spp. at Grassy Hut. Differences in symptom abundances between replicates within Dungrove were minimal. Despite the Permanova+ analysis indicating that provenance effects varied significantly with site and year, there was an overall pattern in a component of the compositional variation among provenances. There was a significant regression of home-site altitude with provenance averages on the first CAP axis of the ordination maximizing provenance differences (Fig. 2.2). Similarly, despite no significant differences between provenances in symptom richness, within Tasmanian provenance means decreased with home-site altitude, while total symptom abundance increased with home-site altitude (Fig. 2.2, Table 2.3).

2.4 Discussion

This study shows that genetic effects are small relative to known drivers of community variation such as year and environment in two eucalypt systems. Site differences explained 3 times the variation of the genetic main effects in *E. globulus*, while year and site both explained approximately 6 times the variation in *E. pauciflora*. The influence of site in this study is similar to that shown in other forest tree systems. For example, environment accounted for approximately 4 times the variation in arthropod species richness than that of the genetic main effects in *Quercus robur* (Tack and Roslin 2011), while accounting for approximately 2 times the variation in fungal pathogen and insect herbivore community structure in *Populus angustifolia* (Busby *et al.* 2014) and *Betula pendula* (Silfver *et al.* 2014), respectively. While the influence of environmental differences is relatively consistent between forest tree systems, fewer studies have examined the impact of yearly differences on community variation relative to genetic and environmental effects. Studies in *P. angustifolia* show little impact of year on fungal pathogen community structure in comparison to genetics and environment (Busby *et al.* 2014) and a highly repeatable structure in the arthropod community across multiple years (Keith *et al.* 2010). This is in contrast with *E. pauciflora* in the present study where the impact of yearly differences is comparable to that of site, accounting for 6 times the variation in the arthropod and fungal community than that of genetic main effects. The impact of year in this study is likely due to a higher infestation of arthropod

larvae in 2015, with the average abundance 4 times that of 2014 for both *Paropsisterna spp.* and *Lepidoptera* larvae.

Despite their relatively small effect size, this study reveals a degree of stability through different colonization events not only in the existence of genetic effects across environments and time, but the pattern of these effects. In *E. globulus*, the main direction of sub-race variation in dependent communities at both sites is associated with a latitudinal trend. The main differences in dependent community composition associated with the difference between mainland and Tasmanian sub-races with the geographically intermediate Bass Strait island sub-races tending to be intermediate in community composition. This pattern of sub-race variation on the dependent arthropod and fungal community in *E. globulus* was first reported in Barbour *et al.* (2009c), demonstrating a spatial and temporal stability to the community genetic impact of the phenotypic differences between these sub-races. While the pattern of variation in the dependent canopy community is consistent between the studies, and sites within the present study, at this broad level the organisms comprising the community may differ between sites and years depending on factors such as colonization sources and history. While there were a few identifiable symptoms consistent between the studies (ie. *Paropsisterna spp.*, *Gonipterus spp.* and *Teratosphaeria spp.*), *Teratosphaeria* spp. was the only one contributing to community level differences among sub-races in both studies. The genus *Teratosphaeria* (previously *Mycosphaerella*) is a complex of fungal pathogens known to have substantial impacts on *Eucalyptus* tree species (Milgate *et al.* 2005a). Different herbivore and pathogen profiles of the sub-races of *E. globulus*, has implication for pest and disease management in production forestry, and highlights the potential for provenance translocations for conservation/restoration purposes to impact biodiversity values.

The differences in home-site latitude among sub-races of *E. globulus* are associated with environmental differences, for example home-site temperature increases northward (Hamilton *et al.* 2013). The latitudinal variation among sub-races in the composition of the colonizing community in these common garden trials is also underlain by significant genetic-based differences among sub-races. Mainland and Tasmanian *E. globulus* sub-races differ in neutral molecular markers consistent with barriers to gene flow (Jones *et al.* 2013), as well as their quantitative genetics. Common garden trails have revealed genetic-based latitudinal clines in

numerous phenotypic traits from wood (Stackpole *et al.* 2011) and foliar chemistry (Gosney *et al.* 2016; O'Reilly-Wapstra *et al.* 2013b), to disease susceptibility (Hamilton *et al.* 2013), with many traits showing evidence of diversifying selection (Dutkowski and Potts 2012; Gosney *et al.* 2016; O'Reilly-Wapstra *et al.* 2013b). For example, cuticular wax compounds showing a latitudinal cline and signals of diversifying selection (Gosney *et al.* 2016) have been linked to arthropod and fungal community variation in *E. globulus* in a recent study involving one of the sites in the present study (Chapter 5). Thus, a component of the extended genetic effects detected in *E. globulus* may be a direct or indirect consequence of adaptive differences associate with home-site climate. However, this could dependent on the stability of phytochemistry and dependent organisms across sites and years. For example, the FPC compound sideroxylonal which has been linked as a mechanism driving genetic-based arthropod herbivory in eucalypts (Andrew *et al.* 2007; Henery *et al.* 2008) has shown a significant genetic x environment interaction at the population level in *Euclayptus tricarpa* (Andrew *et al.* 2010).

The main direction of provenance variation in the dependent community of the Tasmanian *E. pauciflora* is associated with an altitudinal cline with an increasing total abundance of symptoms and decreasing symptom richness with increasing home site altitude of provenances. Genetic-based altitudinal clines in functional traits among populations have been reported for both mainland (Green 1969; Pryor 1956; Slayter and Morrow 1977) and Tasmanian (Gauli *et al.* 2015) *E. pauciflora.* In the Tasmanian populations of *E. pauciflora*, glasshouse progeny trials have shown the main direction of population differentiation in seedling morphology is closely associated with variation in home site altitude (Gauli *et al.* 2015). Numerous functional traits in *E. pauciflora* are associated with provenance home-site altitude with many showing signals of diversifying selection (Gauli *et al.* 2015). For example, seedling leaf color and stem rugoseness showed greater population divergence than expected based on neutral marker divergence; and with increasing home-site altitude leaves were darker and the density of oil glands on the seedling stems increased. The population divergence in these functional traits may have extended effects on the dependent community, however, this has not been explored in *E. pauciflora*. In other plant systems, variation in leaf color has been linked to dependent organisms (Karageorgou and Manetas 2006; Kursar and Coley 1992; Smith 1986). For example, red leaves in the forest tree *Quercus*

coccifera show less leaf area loss to insect herbivory than that of green leaves, which may be in part be related to total phenolic content (Karageorgou and Manetas 2006).

The development of different dependent communities on populations sourced from different environments is clearly underlain by significant genetic differentiation in functional traits in both the *E. globulus* and *E. pauciflora* systems studied. Such extended genetic effects could be an indirect consequence of adaptation to the abiotic components of the environment which differ along these latitudinal and altitudinal gradients, and/or a direct consequence of different evolutionary histories of biotic interactions as may be expected with a geographic mosaic of coevolution (Thompson 2005). In the case of different biotic interactions, there is some evidence for pathogen-imposed selection in *E. globulus*. Sub-race differences in the susceptibility of juvenile foliage to damage by *Teratosphaeria* pathogens show a latitudinal cline in *E. globulus* (susceptibility increased southward as in the present study), and a signal of diversifying selection consistent with pathogen-imposed selection in areas of high disease risk (Hamilton *et al.* 2013). These fungal pathogens cause a leaf disease which can inhibit photosynthesis (Pinkard and Mohammed 2006), cause severe defoliation (Carnegie *et al.* 1994), and reduce tree growth (Milgate *et al.* 2005a). There is also evidence that such disease may have extended effects to other community members (Jones *et al.* 2002). In the case of *E. pauciflora*, there is some evidence enemy communities may vary with altitude, with Burdon and Chilvers (1974) reporting an increase in leaf area loss associated with insect and fungal damage with increasing altitude in wild mainland populations. However, given that a similar trend, in terms of symptom abundance was observed in the common garden experiment (Fig. 2.2), the possibility this observation in wild could confound differences in tree susceptibility cannot be dismissed. Nevertheless, regardless of the cause the present study argues that translocation of germplasm (sub-races or populations) from different home-site environments likely have different extended genetic effects, which may or may not be linked with adaptation depending upon the evolutionary history of home site biotic interactions.

In conclusion, the present study highlights the possibility that focal tree adaptation to major environmental gradients associated with latitude and altitude may have extended genetic effects on dependent communities. Such extended effects may impact community composition, abundance and richness. While such extended genetic effects may only account for a small

component of the variation in dependent communities, they are detectable and appear relatively stable in shaping the trajectory of the community development following provenance translocation. There have been few community genetic studies of inter-provenance variation within species, yet for foundation species such as trees the extended effects of provenance variation to the community and ecosystem levels has implication for provenance choice in ecological restoration, conservation strategies, and management responses to global climate change such as assisted migration and assisted gene flow. In these areas, the focus has to-date been on tree adaptation, with little regard to the extended consequences of provenance choice for translocation and their feedbacks (Bucharova 2016). Outside of *Eucalyptus*, only *Quercus petraea* and *Betula pendula* have shown the influence of provenance variation on the dependent community (Heimonen *et al.* 2017; Sinclair *et al.* 2015). In the case of *Eucalyptus*, our results provide evidence indicating the importance of provenance in structuring dependent communities, with stable extended genetic effects on the dependent arthropod and fungal community shown in multiple systems.

2.5 Acknowledgments

We thank Paul Tilyard, and Justin Bloomfield for collection of the samples involved in this study, Tim Wardlaw and Caroline Mohammad for help in the identification of symptoms, Peter Harrison for symptom assessment. We also thank Forestry Tasmania (particularly Dean Williams), Peter Bignell, and Peter and Anne Downie for access to the trials. This study is part of a PhD undertaken by Benjamin Gosney, supported by an International Postgraduate Scholarship provided by the University of Tasmania and funded by the Australian Government. Data collection was funded by Australian Research Council Discovery (DP0773686) and Linkage (LP120200380 in partnership with Greening Australia) grants.

Chapter 3. Genetic and ontogenetic variation in an endangered tree structures dependent arthropod and fungal communities[1]

Abstract

Plant genetic and ontogenetic variation can significantly impact dependent fungal and arthropod communities. However, little is known of the relative importance of these extended genetic and ontogenetic effects within a species. Using a common garden trial, we compared the dependent arthropod and fungal community on 222 progeny from two highly differentiated populations of the endangered heteroblastic tree species, *Eucalyptus morrisbyi*. We assessed arthropod and fungal communities on both juvenile and adult foliage. The community variation was related to previous levels of marsupial browsing, as well as the variation in the physicochemical properties of leaves using near-infrared spectroscopy. We found highly significant differences in community composition, abundance and diversity parameters between eucalypt source populations in the common garden, and these were comparable to differences between the distinctive juvenile and adult foliage. The physicochemical properties assessed accounted for a significant percentage of the community variation but did not explain fully the community differences between populations and foliage types. Similarly, while differences in population susceptibility to a major marsupial herbivore may result in diffuse genetic effects on the dependent community, this still did not account for the large genetic-based differences in dependent communities between populations. Our results emphasize the importance of maintaining the populations of this rare species as separate management units, as not only are the populations highly genetically structured, this variation may alter the trajectory of biotic colonization of conservation plantings.

[1] Published as: Gosney BJ, O'Reilly-Wapstra JM, Forster LG, Barbour RC, Iason GR, Potts BM (2014) Genetic and ontogenetic variation in an endangered tree structures dependent arthropod and fungal communities. *PLoS ONE* 9(12): e114132. doi:10.1371/journal.pone.0114132.

2.1 Introduction

Plant genetic variation has an extended effect on dependent community and ecosystem processes in many differing systems (Whitham *et al.* 2012). Foundation tree species have been a focus of research due to their disproportionate influence on the surrounding environment (Whitham *et al.* 2006). Trees can act as community and ecosystem drivers, creating locally stable environmental conditions for communities to develop and ecosystem processes to operate, making them important in conservation and restoration research (Ellison *et al.* 2005; Whitham *et al.* 2010). Conservation and restoration research has mainly concerned ecological issues, such as which species and what type of species mixes to include and what kind of regeneration planning to implement. Less research has focused on the importance of genetic factors in forest restoration (Hobbs 2007). The influence of genetic variation across the range of a species has become an important issue for conservation and restoration planning, particularly as there is increasing interest in species and provenance translocations to account for climate change (Aitken and Whitlock 2013). While most work has focused on adaptive impacts of translocation, little is known of the possible effects of such translocations on dependent communities (Frascaria-Lacoste and Fernandez-Manjarres 2012).

The effects of genetic variation on foundation trees can occur at multiple genetic scales. Variation in extended genetic effects on dependent communities can occur across geographic races (Barbour *et al.* 2009c), populations within races, families within populations and clones (Whitham *et al.* 2006). Strong extended genetic effects have been well documented in the North American *Populus* system, which established a framework for community and ecosystem genetics research using clonally replicated tree genotypes planted in common gardens (Whitham *et al.* 2006). Recent literature has raised other factors, such as environmental variation, as being important in community composition, which may outweigh genetic-based effects (Busby *et al.* 2014; Tack *et al.* 2012). Another factor that has rarely been examined is developmental change.

In addition to genetic variation, developmental changes in plants are known to affect herbivory and physicochemical processes (Holeski *et al.* 2012). Most plants are under some form of genetically controlled developmental change (ontogenetic variation) in phenotypic traits (Zotz *et al.* 2011). Such ontogenetic variation leads to changes in gene expression through development

(Maherali *et al.* 2009) and can result in dramatic changes in morphology through an individual's life stages (Zotz *et al.* 2011). Heteroblasty is one such case whereby the leaves of a plant can change markedly from juvenile to adult ontogenetic stages (Zotz *et al.* 2011). Heteroblasty is no better illustrated than in the genus *Eucalyptus*, where significant differences in morphology and physicochemical properties occur between juvenile and adult foliage of many species (Wiltshire *et al.* 1998).

The tree genus *Eucalyptus* dominates the landscape of Australia, comprising nearly 900 taxa (Centre for Plant Biodiversity Research and Slee 2006). A single tree can support a large abundance and diversity of organisms, influencing ecological processes (Abbott and Wills 2001). While there have been numerous studies of tree genetic effects on single organisms in many eucalypt species (Dungey *et al.* 1997; Rapley *et al.* 2004a), extended effects of intra-specific genetic variation on dependent communities (Barbour *et al.* 2009c) and ecosystem processes (Bailey *et al.* 2011) have only been studied in one widespread species, *Eucalyptus globulus*, due to its commercial importance in the plantation industry. However, the genus *Eucalyptus* has many rare species, often occurring naturally as small isolated populations which can be highly genetically differentiated (Byrne 2008). Understanding how communities respond to genetic variation in rare species will allow for improved management of the species and their dependent communities (Hersch-Green *et al.* 2011; Whitham *et al.* 2010). This study examines the dependent arthropod and fungal community of the heteroblastic tree species *Eucalyptus morrisbyi*, a rare eucalypt species restricted to just four small populations in Tasmania. This species is of particular relevance due to the ongoing activity in *in situ* and *ex situ* conservation (Jones *et al.* 2005).

In this study, we examined the genetic and ontogenetic variability of *E. morrisbyi* in relation to dependent arthropod and fungal communities. We assessed the two main populations of *E. morrisbyi* in a single common environment trial, addressing four main questions: First, does genetic and ontogenetic variation influence community composition? Second, what is the relative influence of these genetic and ontogenetic effects? Third, what are the key organisms responsible for community responses? Finally, what are the mechanisms driving differences in communities and what is the impact of previous marsupial browsing?

3.2 Materials and methods

3.2.1 Ethics statement

The sampling performed in this study of the rare species *E. morrisbyi* was undertaken under a Tasmanian Department of Primary Industries, Parks, Water and Environment permit (TLF14060). Permission to access the trial was obtained from Forestry Tasmania.

3.2.2 Study species

Eucalyptus morrisbyi is endemic to the island of Tasmania, Australia. The species consists of four populations, two main populations and the other two with only a few individuals (Jones *et al.* 2005). The two main populations of the species, Risdon Hills (42°49'S, 172°20'E; 81 mature trees; RH) and Calverts Hill (42°56'S, 147°31'E; 1915 mature trees; CH), are separated by nearly 20km and exhibit high molecular genetic variability both between and within populations (Jones *et al.* 2005). As part of conservation efforts for the species, *ex situ* plantings at multiple sites capture a large array of the genetic diversity in both the main populations. These plantings have recently revealed genetic-based variation in *Trichosurus vulpecula* (brushtail possum) browsing with the CH population being heavily browsed, causing poor growth and delayed ontogenetic transition to the adult foliage (Mann *et al.* 2012). This genetic-based resistance is related to key physicochemical traits differing between the populations (Mann *et al.* 2012).

3.2.3 Common environment trial

The common garden field trial used for the study consisted of families from the two main native populations of *E. morrisbyi*, Calvert's Hill and Risdon Hill. It was established at Geeveston, Tasmania, Australia (43°09'S, 146°51'E) in October 1999 from seedlings grown from open-pollinated seed from maternal trees in the natural populations. Seed from each maternal tree is a family and 30 families from each of the CH and RH populations were planted in a randomized block design (Mann *et al.* 2012). A total of 480 trees were planted in eight replicated blocks of alternating rows of CH and RH individuals, with each family represented as a single-tree plot within each replicate. We assessed population and family level variation in the dependent arthropod and fungal community and physicochemical properties of leaves from 222 progeny (129 CH; 93 RH) in this trial with juvenile foliage. While most of the CH trees were in the juvenile leaf stage seventy-nine of the individuals from the RH population displayed both juvenile and adult

foliage, allowing for assessment of the effect of ontogenetic variation (ie. different foliage types) within this population.

3.2.4 Community assessment

Trees were sampled on May 22nd and 23rd of 2007. Samples for community assessment were collected as single branches cut from the mid canopy of the northeast aspect of individual trees. Leaves were removed from the branches in the laboratory, total dry weight obtained, and number of leaves estimated. Following previous approaches (Barbour *et al.* 2009c; Tack and Roslin 2011), dependent organisms were identified based on the presence of their causal symptom on damaged leaves. This technique is widely used in herbivore and community studies (Lawrence *et al.* 2003; O'Reilly-Wapstra *et al.* 2002; Wise 2007) as an alternative to live organism assessment. While it is a useful technique for such large studies, this approach does have conceptual limitations. For example, symptom abundance and richness may be unrelated to actual organism abundance and richness (Bito *et al.* 2011). A single identified symptom may be caused by many individuals or may be covering previous damage by an unidentified organism. In contrast, a recent study has shown that leaf-chewer symptoms can be used to interpret insect richness and composition as they were found to be correlated in both the fossil record and living forests (Carvalho *et al.* 2014). In this study, organisms identifications based on damage type were classified to species level where possible and genus level where not, based on publications and previous field and lab observations (see Appendix B Table 3.S1 and Fig. 3.S1). A few damage types were classified as unknown due to lack of observable evidence of causal organisms performing the damage. The raw abundance scores for putative causal organisms used in the analysis of this study represent the percentage of leaves affected by a given symptom from a ten-gram sample of leaves.

3.2.5 Assessment of physiochemical properties of leaves

Samples for assessment of the physicochemical properties of leaves were collected in the same manner as the community samples, with single branches cut from the mid canopy of the northeast aspect of individual trees. Ten to thirty fully expanded leaves from the sampling years growth were collected from each branch, freeze-dried and analyzed for their physicochemical differences using near-infrared spectroscopy (NIR). The variation in the NIR spectra of leaves were used to quantify the holistic physiochemical profile of leaves without identifying their individual physical

components (Munck 2007; O'Reilly-Wapstra *et al.* 2013a) for inference of potential mechanisms driving genetic-based community variation. Freeze-dried leaves from each sample were scanned using a Bruker MPA FT-NIR spectrometer with a fiber-optic probe. Spectral wavelengths for each sample were obtained from the 780-2500 nm range at a $4cm^{-1}$ resolution. Five leaves per sample were scanned four times on either side of the midrib toward the tip and base of each leaf, resulting in forty scans per sample. Recorded wavelengths for each sample are the means of all scans from the five leaves (8 scans per leaf; 40 scans total). The 220 wavelengths obtained from the NIR analysis were reduced to 20 principal components, which were used to summarize the variation in physicochemical properties (O'Reilly-Wapstra *et al.* 2013a), these will be referred to as NIR PCs from here on in. Prior to conversion of wavelengths, the NIR spectra was narrowed to 1300-2300 nm due to minimal variation in the spectra from 780-1300 nm and underwent standard smoothing procedures using 11 smoothing points with Unscrambler (Version 9.6; CAMO ASA, Oslo, Norway).

3.2.6 Trichosurus vulpecula *browsing assessment*

Mann *et al.* (2012) showed clear genetic-based differences in *T. vulpecula* browsing susceptibility of *E. morrisbyi* trees assessed in the same experimental common environment trial used in this study. Variation in *T. vulpecula* damage was linked to differences in key chemical and physical foliar properties. Due to the degree of impact that this marsupial herbivore has on the trees, it is possible they influence the dependent arthropod and fungal community. Hence, in this paper we examine the relative influence that previous browsing by *T. vulpecula* has had on the dependent community. Assessment of *T. vulpecula* browsing on each tree across the trial was performed in 2005 (Mann *et al.* 2012). Damage scores were visually estimated for each tree on a five point scale based on their percent damage to total leaf area: 0 = no damage, 1 = 1-25%, 2 = 26-50%, 3 = 51-75% and 4 = greater than 75%.

3.2.7 Multivariate Community Analysis

The influence of genetic and ontogenetic variation on dependent arthropod and fungal community composition were tested separately in Primer (version 6.1.3; Roborough, Plymouth, UK) using two models (model I & II; Table 3.1), with population and foliage type treated as fixed effects and replicate (spatial variation within the trial) and family within populations treated as random effects.

The weighted community data were standardized to the unit maxima for each symptom in order to reduce disproportionate effects of highly abundant symptoms (Quinn and Keough 2002). A Bray-Curtis dissimilarity matrix based on the standardized symptom data was used to represent community composition dissimilarity amongst samples. The significance of genetic and ontogenetic variation was tested using a permutational multivariate analysis of variance (PERMANOVA - Anderson *et al.* 2008) of the dependent arthropod and fungal community within the Bray-Curtis dissimilarity matrix. A canonical analysis of principal components (CAP) (Anderson and Willis 2003) of the community Bray-Curtis dissimilarity matrix of all juvenile and adult samples, maximizing group differences, was performed to provide a visual interpretation of the community variation between both populations and foliage types.

The relative influence of physicochemical properties of leaves on the dependent community associated with genetic and ontogenetic variation were tested by including the twenty NIR PCs as covariates in their respective PERMANOVA analyses with sums of squares (SS) calculated sequentially (Type I SS). Spatial variation within the trial (replicate) was accounted for first in all analyses in order to remove the effect before the main effects of interest using models III and IV (Table 3.1). Similarly, a test for the influence of *T. vulpecula* browsing was conducted, but only for the genetic model, by including browsing scores with physicochemical properties of leaves as covariates. Three separate analyses of these influences on the dependent community were performed using models V, VI and VII (Table 3.1).

Table 3.1. Mixed-models used in the study for analysis of significance and the partitioning of effect sizes.

Analysis model	Model description
Model I	$y = \mu +$ *Replicate* + **Population** + *Family(Population)* + *Residuals*
Model II	$y = \mu +$ *Replicate* + **Foliage Type** + *Residuals*
Model III	$y = \mu +$ *Replicate* + NIR PCs + **Population** + *Family(Population)* + *Residuals*
Model IV	$y = \mu +$ *Replicate* + NIR PCs + **Foliage Type** + *Residuals*
Model V	$y = \mu +$ *Replicate* + Browsing + NIR PCs + **Population** + *Family(Population)* + *Residuals*
Model VI	$y = \mu +$ *Replicate* + NIR PCs + Browsing + **Population** + *Family(Population)* + *Residuals*
Model VII	$y = \mu +$ *Replicate* + NIR PCs + **Population** + *Family(Population)* + Browsing + *Residuals*

Fixed effects for each model are in boldface, random effects and residuals are italicized and covariates are underlined. Ordering of effects and covariates in each model are those used in the Type I SS analyses described in the methods.

The proportion of variation influencing dependent community composition was calculated using components of variation obtained from the PERMANOVA analysis for each effect and covariate, following Anderson *et al.* (2008). Total variance in each model was calculated by adding these components of variation and the residual variance, with negative components treated as zero (Fletcher and Underwood 2002). The twenty components of variation obtained for physicochemical properties of leaves were compiled by summing all twenty components to represent their holistic influence on the dependent community. The percent of genetic and ontogenetic variation in dependent community composition accounted for by physicochemical properties of leaves was calculated from the components of variation obtained from the analyses with NIR PCs included and excluded (model I vs. III and model II vs. IV). This was also done to determine the percent variation attributed to genetic variation and physicochemical properties in dependent communities accounted for by *T. vulpecula* browsing (model III vs. V).

3.2.8 Community parameters analysis

Community parameters were calculated for each sample from the weighted symptom scores using the function *diversity* from the package *vegan* in R. The parameters calculated included richness, abundance, Shannon diversity index and Pielou's evenness. Community parameters were analysed for genetic variation using a mixed model (model I) with the significance of random effects tested with the one-tailed likelihood ratio test and the fixed population effect with a Wald-F test. A paired sample t-test was used to analyse variation in community parameters between foliage types. Significance values for population effects and foliage types effects were adjusted to p=0.0125 for community parameters using the Bonferroni method to correct for false discovery rates due to multiple testing (Benjamini and Hochberg 1995). Analyses were performed using the package *asreml* and the function *t.test* from the base *stats* package in R 2.15.3 (R Core Team 2013).

3.2.9 Analysis of individual symptoms

Individual symptoms were analyzed using a nonparametric Kruskal-Wallis test for population differences and a paired sample t-test for foliage type differences on the standardized abundance scores. Only symptoms found on at least ten percent of all trees from their respective data sets were subject to univariate analyses. Significance values were corrected using Bonferroni adjustment of p-values based on the number of organisms analyzed from each data set to account

for multiple testing. Symptoms found significant in the univariate analyses were then used to determine which symptoms were influencing the variation in community composition between populations and foliage types. Univariate analyses of symptoms were undertaken using the functions *kruskal.test* and *t.test* from the base *stats* package in R 2.15.3.

Significant symptoms were ranked based on their univariate Kruskal X^2 or t-test values, from highest to lowest (1=highest, 2=lowest). Following the principles of stepwise multiple regression (Hocking 1976), these symptoms were sequentially excluded from calculations of the Bray-Curtis dissimilarity matrices used in multivariate PERMANOVA community analyses (models V and IV) until the significance of population and foliage type effects were lost in their respective analyses. Following the loss of significance of the effect of interest, the symptoms removed were individually included back into the PERMANOVA community analyses to validate their significance. The final resulting exclusions were those found to be the main contributors to variation in community composition between populations and foliage types.

3.3 Results

3.3.1 Genetic variation in foliar organism responses

A total of 60 symptoms of arthropod (primarily herbivorous arthropods) and fungal species were identified on the sampled juvenile leaves between the two populations in the common garden trial. We found highly significant differences in arthropod and fungal communities on the juvenile leaves from RH and CH populations (PERMANOVA model I, Pseudo-$F_{1,221}$=10.9, $p<0.001$), however the family within population effect was not significant (PERMANOVA, Pseudo-$F_{53,221}$=0.99, p=0.557). This significant variation is also shown in the ordination space derived from the CAP analysis with the two populations clearly separated (Fig. 3.1). Spatial variation within the trial also significantly affected community composition (PERMANOVA, Replicate: Pseudo- $F_{5,221}$=1.43, p=0.005), however, components of variation obtained from the PERMANOVA analysis showed that its influence was small (1.2% of total variation) when compared with the population (genetic) effect (9.5%). To test if the difference on the juvenile foliage between populations was due to an effect of the presence of adult foliage on the RH population, we tested for a difference in community composition on the juvenile foliage between RH trees with only juvenile foliage and those with both juvenile and adult foliage. This difference

was not significant (PERMANOVA, Pseudo-$F_{1,92}$=1.15, p=0.28), indicating the population differences were not due to the presence of adult foliage.

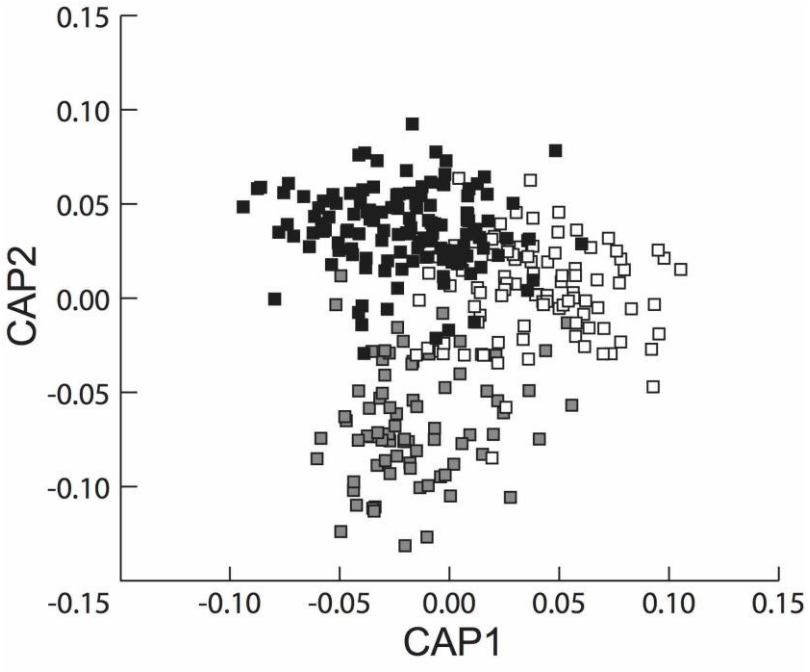

Fig. 3.1. Canonical plot summarizing the variation in foliar community composition between populations and ontogenetic stages of *E. morrisbyi*. The constrained canonical analysis was undertaken on the principal coordinates derived from the Bray-Curtis dissimilarity matrix of all samples with the three a priori groups indicated summarizing the multivariate community variation between *E. morrisbyi* populations and foliage types. Each point represents an individual sample within the common garden trial. Pairwise Permanova+ analysis showed significant variation between all three groups (CH juvenile – RH juvenile, t=3.6, p=<0.001; RH juvenile – RH adult, t=3.6, p=<0.001; CH juvenile – RH adult, t=5.0, p=<0.001). RH adults are grey squares, RH juveniles are white squares, and CH juveniles are black squares.

Two other factors could also influence variation in the arthropod and fungal community: NIR spectra variation (physicochemical properties) and variation in possum browsing. We tested whether population alone accounted for variation in the community after accounting for these two factors. By comparing the reduction in the percent variation attributed to population differences before and after including the NIR spectra principal components as covariates, we showed physicochemical properties of leaves accounted for 18% of the differences in community composition between populations. However, there were still significant differences in dependent

community composition between populations (PERMANOVA model III, Pseudo-$F_{1,221}$=4.3, p<0.001). On juvenile leaves, 5.7% of the variation in dependent community composition was explained by physicochemical properties of leaves, population 7.8% and spatial variation (ie. replicate) within the trial 1.3%. *Trichosurus vulpecula* browsing also significantly influenced variation in dependent community composition (PERMANOVA model V, Pseudo-$F_{4,221}$=7.6, p<0.001) when accounted for first in the analyses. By comparing the reduction in the percent variation attributed to physicochemical properties of leaves and population differences before and after including *T. vulpecula* browsing as a covariate, it was shown that *T. vulpecula* browsing removed 44% of the variation in physicochemical properties (NIR spectra principal components) and 9.5% of the differences in community between populations. The browsing effect was still significant (PERMANOVA model VI, Pseudo-$F_{4,221}$=1.8, p=0.018) when placed after the principal components derived from the NIR spectra of juvenile foliage, indicating physicochemical properties do not account fully for the effect *T. vulpecula* browsing on community differences between populations. However, significance was lost (PERMANOVA model VII, Pseudo-$F_{4,221}$=1.0, p=0.455) when browsing was accounted for last in the analysis, indicating that the community effect of *T. vulpecula* browsing can be explained by a combination of population and physicochemical differences. The genetic effect (population) remained highly significant in each model. Overall, *T. vulpecula* browsing explained 2.6% of the variation in dependent community composition, physicochemical properties of leaves 2.8%, population 7.1% and spatial variation within the trial 1.3% (Fig. 3.2a – model V).

When we examined individual community parameters as opposed to overall community differences, we also found significant differences between populations. All community parameters investigated (richness, abundance, Shannon-Weiner diversity index, and Pieolou's evenness) were found to be significantly different between populations based on juvenile leaf samples after a Bonferroni adjustment to p=0.00125 (Table 3.2). On average, more symptoms (richness) were found on trees from the RH population (Fig. 3.3), while more overall damage (abundance) was found on trees from the CH population (Fig. 3.3). Overall species diversity and evenness was greater on the juvenile foliage of the RH than CH population (Figure 3.3).

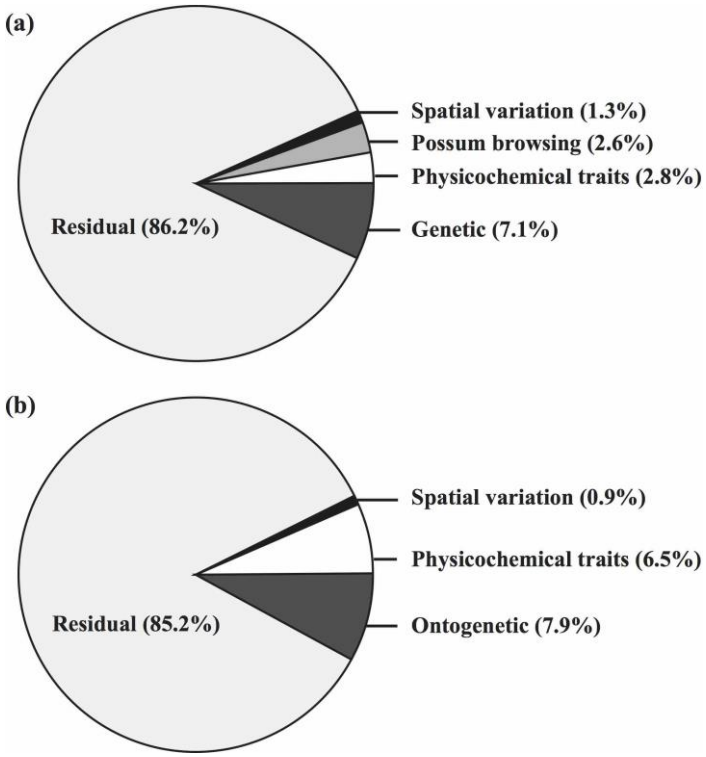

Fig. 3.2. Pie charts summarizing the partition of variation in the dependent community Bray-Curtis dissimilarity matrices for (a) populations and (b) foliage types using Permanova+. The genetic effect represents only the variation between populations, as the family within population variation was not significant.

Table 3.2. Tests for the differences in community parameters between populations, families within populations and foliage types of *E. morrisbyi*.

	Juvenile Foliage				Juvenile and Adult Foliage	
	Population (Fixed)		Family(Pop.) (Random)		Foliage Type	
Community Parameter	Wald-F	P	$\chi 2$	P	t	P
Abundance (log10)	**18.3**	**<0.001**	0	1	-0.9	0.393
Richness	**27.4**	**<0.001**	0.4	0.53	**-12.7**	**<0.001**
Shannon-Weiner diversity	**76.9**	**<0.001**	0	1	-1.8	0.081
Peilou's evenness	**24.0**	**<0.001**	0	1	-1.8	0.071

Statistical significance in community parameters between populations and family within populations was tested with generalized linear mixed model (model I). Replicate was not significant for any community parameter. Statistical significance in community parameters between foliage types was tested with paired sample t-tests. Foliage type t-values represent the mean difference in adult foliage minus juvenile foliage (A-J). Transformations, if necessary, are given in parentheses. Significant values after Bonferroni adjustment of p-values are in boldface.

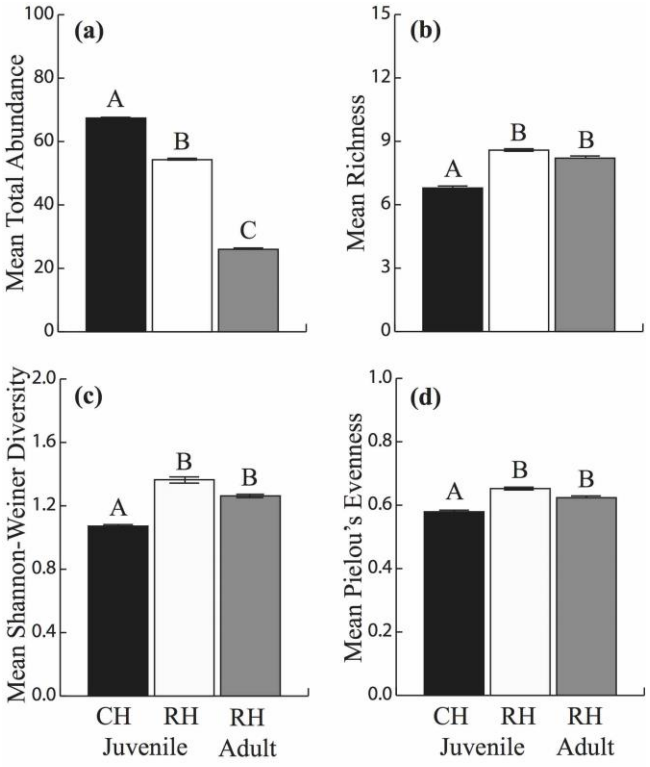

Fig. 3.3. Mean community parameters for populations (CH and RH) and foliage types (juvenile and adult of RH) of *E. morrisbyi*. (a) Total abundance of all identified organisms (abundance), (b) the number of identified organisms (richness), (c) Shannon-Weiner diversity index, and (d) Pielou's evenness. Different letters above the bars represent significant differences between groups based on a linear mixed model for population differences and paired sample t-tests for foliage type differences. Error bars represent $\pm$ 1 SE. All community parameters were calculated from the weighted abundance scores. Differences in community parameters between CH juvenile and RH adult foliage were not tested.

Of the 60 symptoms identified on juvenile foliage forty-seven of these were found to be common between populations, but only twenty-one were found to occur on over ten percent of the juvenile foliage samples. A total of eight of the twenty-one symptoms found on more than ten percent of the samples were significantly different between populations after a Bonferroni adjustment of significance to p=0.002 (Table 3.3), based on the twenty-one symptoms analyzed. Stepwise exclusions of these eight symptoms from the PERMANOVA community analysis (model V) showed only two symptoms of the original sixty were responsible for the variation in community composition detected between the juvenile foliage of the CH and RH populations. These highly influential symptoms were the fungal pathogen *Teratosphaeria spp*. and adult leaf beetle *Paropsisterna spp*., which both had a greater abundance on the CH population (Fig. 3.4).

Table 3.3. Significance of arthropod and fungal causal organisms found responsible for the variation in dependent community composition between populations and foliage types of *E. morrisbyi*.

	Juvenile Foliage		Juvenile and Adult Foliage	
	Population		Foliage Type	
Putative Causal Organism	Kruskal (χ2)	P	t	P
Teratospharia spp. (sqrt)	**68.6**	**<0.001**	**-5.2**	**<0.001**
Paropsisterna spp., adult (sqrt)	**13.8**	**<0.001**	**-9.5**	**<0.001**
Gonipterus scutellatus, larvae (sqrt)	29.4	<0.001	**-4.7**	**<0.001**
Pachysacca samuelii	18.7	<0.001	**-5.5**	**<0.001**
Hymenoptera spp. 3 (sqrt)	11	<0.001	**-2.7**	**0.008**
Acrocercops laciniella (sqrt)	4.1	0.043	**-6.7**	**<0.001**
Ctenarytaina eucalypti	2.8	0.095	**-5.5**	**<0.001**
Hyalinaspis spp. (sqrt)	0.6	0.428	**-3.3**	**0.002**

Organisms listed were those found to be the main symptoms influencing variation in community composition between populations and foliage types based on their stepwise elimination from the Permanova+ analysis. Causal organisms found to significantly influence the variation in dependent community composition between populations and foliage types from this step-down analysis are presented in boldface. Univariate tests of the significance in organism abundance are shown. Between populations this was tested with nonparametric Kruskal-Wallis tests. Statistical significance in organism abundance between foliage types was tested with paired sample t-tests. Foliage type t-values represent the mean difference in adult foliage minus juvenile foliage (Adult-Juvenile). Transformations, if necessary, are listed.

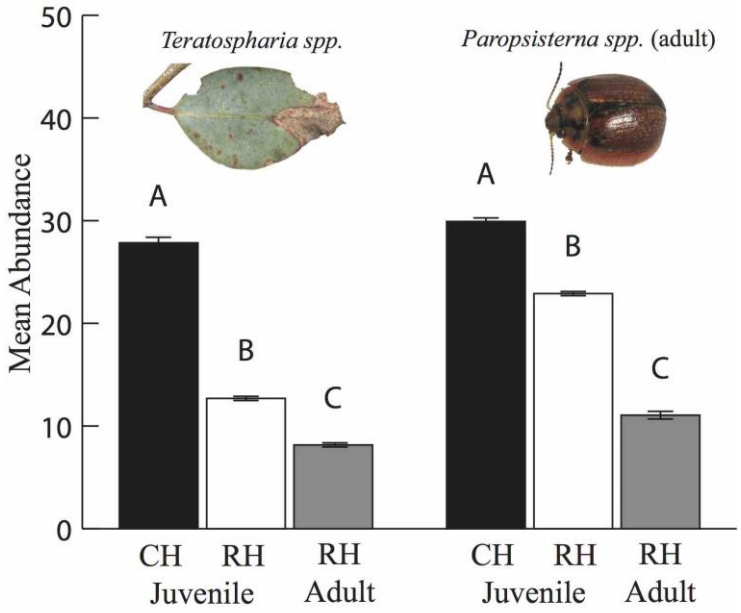

Fig. 3.4. Mean abundance per sample of the two main symptoms driving community differences (fungal pathogen *Teratosphaeria spp.* and the leaf beetle *Paropsisterna spp.*) between populations (CH and RH) and foliage types (juvenile and adult of RH) of *E. morrisbyi*. Different letters above the bars represent significance between groups based on Kruskal-Wallis tests for population differences and paired sample t-tests for foliage type differences on the standardized abundance scores. Variation in causal organism abundances was not tested between CH juvenile and RH adult foliage. Error bars represent $\pm$ 1 SE. Photographs of organisms taken by L. Forster.

3.3.2 Ontogenetic variation in foliar organism responses

A total of 60 symptoms of dependent arthropod and fungal species were identified on the RH population samples having both juvenile and adult foliage. There were highly significant differences in dependent communities on juvenile and adult leaves of the RH population (PERMANOVA model II, Pseudo-$F_{1,157}$=12.92, p<0.001). This difference is clearly seen in the ordination space derived from the CAP analysis (Fig. 1). Spatial variation in the trial was also found to be a significant factor in community composition for this model (PERMANOVA, Replicate: Pseudo- $F_{5,157}$=1.25, p=0.029). However, the variance components obtained from the PERMANOVA analysis showed that this influence was small (0.9% of total variation) when compared with the foliage type effect (13%).

The addition of principal components derived from the NIR spectra as covariates in the community PERMANOVA analysis of the ontogenetic samples showed seven of the principal components significantly influenced the dependent community composition. By comparing the reduction in the percent variation attributed to foliage type differences before and after including the NIR spectra principal components as covariates, it was shown they account for 39% of the differences in community composition explained by foliage types. However, even then the foliage type effect remained significant (PERMANOVA model IV, Pseudo-F1,157=2.8, p<0.001). Over all samples, the total influence of physicochemical properties of leaves explained 7.5%, ontogenetic variation 7.9% and spatial variation (ie. replicate) in the trial an insignificant 0.9% of the variation in dependent community composition (Fig. 3.2b).

Of the community parameters investigated, only abundance was found to be significantly different between foliage types after a Bonferroni adjustment of significance to p=0.0125 (Table 3.2). Abundance of symptoms was greatest on juvenile foliage (Fig. 3.3). Of the 60 symptoms identified, forty-four of these were common between foliage types, eight were only found on the adult foliage and eight were only found on the juvenile foliage. Similar to the between populations data set, the majority of symptoms were rare and found on less than ten percent of the leaves. Of the twenty-one symptoms at higher frequencies, eight were significantly different between foliage types with a Bonferroni adjustment of significance to p=0.002 (Table 3.3). Stepwise exclusions of these eight symptoms from the PERMANOVA community analysis (model IV) showed seven of

the original sixty assessed symptoms were responsible for the variation in community composition detected between adult and juvenile foliage types of the RH population (Table 3.3). All seven symptoms responsible for the variation in community composition detected between foliage types were greater on the juvenile foliage of the RH population (only *Teratosphaeria spp*. and *Paropsisterna spp*. presented in Fig. 3.4). An additional analysis of covariance (ANCOVA) showed that the sampling intensity of adult and juvenile foliage (ie. the number of leaves assessed) significantly influenced only four of the symptoms, however, this did not affect the results between foliage types.

3.4 Discussion

Our study shows that both genetic (9.5%) and ontogenetic (13.0%) variation in *E. morrisbyi* significantly influences the composition of arthropod and fungal communities in this rare heteroblastic eucalypt. Both of these factors remained significant even after accounting for physicochemical properties (for both genetic and ontogenetic) and possum browsing (genetic only). The variation explained by genetic effects in this study was solely driven by differences between populations rather than families. Only four plant systems have quantified the size of genetic-based variation on dependent communities (Busby *et al.* 2013; Johnson 2008; Robinson *et al.* 2012; Tack *et al.* 2010; Whitham *et al.* 2006; Zytynska *et al.* 2011). Three of these systems used clonal replicates for their experiments, all of which exhibited high estimates of genetic influence across the systems (*Populus*–32-93%; *Oenothera*–30%; *Quercus*–7-26%). The other system studied the genetic variation found in a natural forest patch and showed less genetic influence (*Brosimum*–3.7-4.8%). The genetic effects found in our study were smaller than those found in other systems, but were still statistically significant. Our low genetic effect compared to other studies may be in part due to the scoring techniques as the other studies have assessed live organisms as opposed to the symptom-based approach used in this study. As mentioned in the methods, the possibility that symptoms may represent multiple organisms cannot be dismissed, which would potentially reduce host genotype specificity in our study.

There are many differences between studies quantifying host genetic effects on dependent communities. Nevertheless, the low effect values observed in our system may relate to the *Brosimum* system in unexpected ways, despite *Brosmium* being evergreen trees located in warmer

tropical climates. The high diversity of trees found in tropical regions (Wright 2002) along with the moderately high plant diversity and numerous *Eucalyptus* species co-occurring in sclerophyll forests (Parsons and Cameron 1974) may reflect why the influence of genetic variation on community composition was lower in the *Brosimum* and current study, respectively. A study of insect herbivores in a tropical lowland forest in New Guinea has shown that host specificity decreases with increasing number of tree species in a forest (Novotny *et al.* 2002). While this has not been studied in the *Eucalyptus* system, the lower relative influence of genetic variation found in this study may be reflecting a greater frequency of generalist herbivores compared to the other systems. This concept may be further supported by the study species being rare, with common garden trials outside of their natural populations. This distance from natural stands would reduce the occurrence of possible specialist herbivores associated with the species.

With regard to the organisms in the community, we found only two, the fungal pathogen *Teratosphaeria spp.* and the leaf beetle *Paropsisterna spp.*, were driving the differences between populations. Genetic variation in the susceptibility to both *Teratosphaeria spp.* (Dungey *et al.* 1997; Milgate *et al.* 2005a) and *Paropsisterna spp.* (Rapley *et al.* 2004a; Raymond 1995) have been well studied in the other *Eucalyptus* systems, with the presence of these organisms having a deleterious effect on plant growth (Loch and Matsuki 2010; Milgate *et al.* 2005a). In contrast, seven organisms were driving community differences between foliage types. The proportion of organisms driving the genetic-based community differences (3-13%) in this study is in contrast to other systems that have found a greater proportion (45%-100%) of community organisms as significantly different between genotypes (Johnson and Argawal 2007; Wise 2007). This could be due to differences in the number of organisms assessed between the studies, with the studies showing greater proportions of significance assessing less than twenty organisms compared to the sixty symptoms identified in this study. As previously discussed, this may also be due to differences in the frequency of generalist and specialist comprising the dependent communities, as one study has shown increased herbivore diversity to be associated with additional generalists (Salazar and Marquis 2012). This may also be reflected in recordings of higher insect herbivory in eucalypts compared to temperate communities of the northern hemisphere (Fox and Marrow 1983), which may also explain the response of organisms to ontogenetic versus genetic differences,

where a generalist herbivore may respond to the more readily discernable morphological and chemical differences between foliage types than the more subtle differences between populations.

Investigation of the mechanisms driving the variation between communities found that previous *T. vulpecula* browsing accounted for 10% of the genetic effect. This could be due to diffuse interactions (Wise 2010) by which preferences of insects is in part affected by *T. vulpecula* browsing. This interaction may be due to direct or indirect effects. Direct effects include direct ecological interference such as *T. vulpecula* eating preferred foliage of certain organisms feeding on the plants at the same time (e.g. competition). Indirect effects could include: (1) browsing causing the retention of juvenile foliage, influencing the abundance and richness of organisms; (2) induced changes in plant chemistry and/or morphology altering the habitat for arthropod and fungal colonization. To date, however, few studies have demonstrated induction of chemical defenses in eucalypts (Gomes De Oliveira *et al.* 2010; Rapley *et al.* 2007), and so other factors may be at play.

We found the variation in physicochemical traits accounted for 15% of the genetic and 37% of the ontogenetic effects explaining community differences. Numerous studies have shown the importance of variation in traits, such as foliar chemistry, as mechanisms influencing herbivory (O'Reilly-Wapstra *et al.* 2004; Wiggins *et al.* 2006). While both *T. vulpecula* browsing and physicochemical traits did explain some of the population effect, there was still a clear genetic effect (7.1%) influencing community variation. This genetic variation may be affecting the community through: (1) traits undetected in NIR spectra; (2) variation in tree architecture (Larson and Whitham 1997); (3) plant growth (Robinson *et al.* 2012); (4) other diffuse interactions, perhaps from a third trophic level including predatory birds and insects (Smith *et al.* 2011).

The influence of genetic-based effects on community composition in this study shows the importance of maintaining the two populations of this species as distinct management units. For example, the choice of just one of these populations for conservation would lead to a different biotic community trajectory compared to the choice of the other population. Such different trajectories would depend on the stability of genetic-based differences across life history stages (O'Reilly-Wapstra *et al.* 2007), and in contrasting environments. Understanding extended genetic

effects in species of conservation concern allows us to examine the evolutionary relationships between the host plant and their herbivores, to better understand the developing community trajectory in newly planted restored forests and to provide insight for managers on how these rare species may be best managed for the future (Vandegehuchte *et al.* 2012; Whitham *et al.* 2010).

3.5 Acknowledgements

We would like to thank Hugh Fitzgerald, Naomi Glancy, James Marthick and Alison Miller for assistance with foliage collection. We would also like to thank Jon Humphreys and Julia Lord for assistance with the NIR spectra and initial data analysis. We thank the Threatened Species Section of the Tasmanian Department of Primary Industries, Parks, Water and Environment for their support of the *E. morrisbyi* plantings and permits. We thank Forestry Tasmania for access to the field site. We would also like to thank the CRC for Forestry for funding support. Grant Iason was supported by the Scottish Government's Rural and Environment Science and Analytical Services Division and a Royal Society of Edinburgh Travel Grant.

Chapter 4: Genetic control of cuticular wax compounds in *Eucalyptus globulus*[2]

Summary

- Plant cuticular wax compounds perform functions that are essential for the survival of terrestrial plants. Despite their importance, the genetic control of these compounds is poorly understood outside of model taxa. Here we investigate the genetic basis of variation in cuticular compounds in *Eucalyptus globulus* using quantitative genetic and QTL analyses.
- Quantitative genetics analysis was conducted using 246 open pollinated progeny from 13 native sub-races throughout the geographic range. QTL analysis was conducted using 112 clonally replicated progeny from an outcross F_2 population.
- Nine compounds exhibited significant genetic variation among sub-races with three exhibiting signals of diversifying selection. Fifty-two QTL were found with co-location of QTL for related compounds commonly observed. Notable among these was the QTL for five wax esters, which co-located with a gene from the KCS family, previously implicated in the biosynthesis of cuticular waxes in Arabidopsis.
- In combination, the QTL and quantitative genetic analyses suggest the variation and differentiation in cuticular wax compounds within E. globulus has a complex genetic origin. Sub-races exhibited independent latitudinal and longitudinal differentiation in cuticular wax compounds, likely reflecting processes such as historic gene flow and diversifying selection acting upon genes that have diverse functions in distinct biochemical pathways.

[2] Published as: Gosney BJ, Potts BM, O'Reilly-Wapstra JM, Vaillancourt RE, Fitzgerald H, Davies NW, Freeman JS (2016) Genetic control of cuticular wax compounds in *Eucalyptus globulus*. *New Phytologist* 209(1), 202-215. doi: 10.1111/nph.13600.

4.1 Introduction

The plant cuticle (the outermost covering of the epidermis) acts as the first barrier of plants to the environment (Riederer and Schreiber 2001). Waxes are an important component of the cuticle, limiting non-stomatal water loss and gas exchange as well as providing protection from ultraviolet radiation, which are key adaptations in the evolution of terrestrial plants (Raven and Edwards 2004; Yeats and Rose 2013). Waxes are also believed to have other protective roles, including protecting the plant from insect herbivores (Brennan and Weinbaum 2001; Tucker *et al.* 2010) and pathogens (Chassot *et al.* 2007; Serrano *et al.* 2014). Aside from its protective roles, the cuticle also plays a central role in plant development by physically establishing organ boundaries, preventing organ fusion (Weng *et al.* 2010). Cuticular waxes, along with cutin (the structurally important class of compounds in the cuticle) are embedded in the matrix of the cuticle and play a pivotal role in cell-to-cell interactions (Buschhaus and Jetter 2010). Waxes may be either epicuticular or intra-cuticular (Buschhaus and Jetter 2010; Samuels *et al.* 2008). Epicuticular waxes occur on the surface of the leaf and are crystalline in structure (Baker 1982; Jeffree and Sandford 1982). Differences in epicuticular wax structure can lead to diverse, and often morphologically identifiable wax phenotypes (e.g. glaucous, glabrous/glossy; Jenks *et al.* 1996; Wirthensohn *et al.* 1999). Intra-cuticular waxes are different in chemical composition to epicuticular waxes and are embedded in the structural cutin of the cuticular layer (Barthlott *et al.* 1998; Buschhaus and Jetter 2010; Riederer and Müller 2006).

Most understanding of the biosynthesis of cuticular wax compounds comes from *Arabidopsis* (Borisjuk *et al.* 2014; Kunst and Samuels 2003; Lee and Suh 2013; Samuels *et al.* 2008). Cuticular waxes are largely derived from very-long-chain (C20-C34) fatty acids and are highly diverse. Modifications in these very-long-chain fatty acids along the wax biosynthetic pathways lead to primary and secondary alcohols, wax esters, aldehydes and alkanes (Kunst and Samuels 2003; Post-Beittenmiller 1996; Samuels *et al.* 2008). The synthesis of these takes place in three stages, and is based on a fatty acid backbone ranging in length from 16 to 34 carbon molecules (C16-C34) (Kunst and Samuels 2003; Samuels *et al.* 2008). It starts with the synthesis of C16 and C18 fatty acids in the chloroplast of the mesophyll tissues, which are then exported to the epidermal cells (Kolattukudy 1996). This is followed by elongation into very-long-chain fatty acids (C20-C34) by microsomal enzymes in the endoplasmic reticulum of epidermal cells (Borisjuk *et al.* 2014; Lee

and Suh 2013; Samuels *et al.* 2008). Finally, these very-long-chain fatty acids are transformed along their respective pathways into aldehydes, alkanes, alcohols, ketones, and wax esters. Both sequential (generating homologous series) and parallel (generating different wax classes) reactions are involved, with two main biosynthetic pathways identified for the esters (Kunst and Samuels 2003; Post-Beittenmiller 1996; Samuels *et al.* 2008). The alkane pathway (often referred to as the decarbonylation pathway) produces aldehydes, alkanes, secondary alcohols and ketones. The primary alcohol pathway (often referred to as the acyl-reduction pathway) produces primary alcohols and esters (Samuels *et al.* 2008). There is also the less common ß-ketoacyl pathway, which produces ß-diketones (Kunst and Samuels 2003; Le Provost *et al.* 2013; Post-Beittenmiller 1996). This pathway has received far less attention than the others due to its absence in the model species, *Arabidopsis* (Zhang *et al.* 2013). Many of the genes involved in the various steps in these biosynthetic pathways have been identified in *Arabidopsis* (Lee and Suh 2013), as well as in crop species such as maize (Sturaro *et al.* 2005), rice (Islam *et al.* 2009), and tomatoes (Mintz-Oron *et al.* 2008).

The amount and composition of cuticular wax can vary greatly both between and within species, and is influenced by both environmental and genetic factors. Early studies of the population differentiation and adaptive significance of cuticular waxes were based on observable variation in wax phenotypes such as degree of stem and foliar glaucousness. For example, marked clinal variation in wax phenotypes has been observed along environmental gradients in many forest trees consistent with cuticular waxes being functionally important (Barber 1955; Hamrick 1976). More recently, variation in the chemical composition of waxes has been demonstrated at the levels of species (Hoffmann *et al.* 2013; Li *et al.* 1997) and populations (Dodd and Poveda 2003; Dodd *et al.* 1998). For example, *Juniperus communis* wax composition was found to vary significantly along an elevation transect with greater mean alkane chain length at the elevation extremes, which was attributed to adaptation to hot summer temperatures at lower elevation and freezing winters at high elevations (Dodd and Poveda 2003). Few studies have used common garden trials to separate environmental and genetic effects. However, in one recent common garden study in *Pinus pinceana*, wax composition was shown to be significantly different between both the three major geographic regions in Mexico (Northern, Central, and Southern) and populations within regions (Ramirez-Herrera *et al.* 2010). Ecotypes of *Arabidopsis thaliana* were also found to vary

significantly in total wax load. However, the relative proportions of different wax compounds were highly conserved among ecotypes (Rashotte *et al.* 1997).

Here we examine the genetic basis of variation in foliar cuticular wax compounds (waxes and embedded flavonoids) within *Eucalyptus globulus*. *Eucalyptus globulus* is a forest tree native to southeastern Australia, and is grown commercially around the world (Dutkowski and Potts 1999). The species is heteroblastic (Hudson *et al.* 2014; James and Bell 2001), and part of a complex of four related species (Jones *et al.* 2011). Natural patterns of genetic variation in *E. globulus* have been well studied, including wood chemistry (Stackpole *et al.* 2011) and foliar secondary metabolites (O'Reilly-Wapstra *et al.* 2013b). There is significant quantitative genetic variation across the geographic range of the species in virtually all traits studied which has been summarized by classification of the native range into 13 geographic races and 20 sub-races (Dutkowski and Potts 1999). In this study, we explore the spatial patterns of genetic variation in cuticular wax compounds, including the phenotypic and genetic relationship between these compounds. We test for a role of diversifying selection in shaping patterns of sub-race differentiation, and identify underlying QTL and putative candidate genes responsible for the genetic variation in these cuticular wax compounds.

4.2 Materials and methods

4.2.1 Experiment 1: Quantitative genetics study

Genetic-based variation in wax chemistry at the sub-race and family within sub-race levels was studied by growing families with known female pedigrees in a replicated common environment field trial (Hamilton *et al.* 2013; O'Reilly-Wapstra *et al.* 2013b); located at Salmon River (41° 01' S, 144° 52' E) in northwest Tasmania, Australia. The trial was established in 2006 using progeny derived from open-pollinated seed (families) from 140 trees sampled across the natural range of *E. globulus*. Full trial details are given in Hamilton *et al.* (2013). In brief, families were planted as single-tree plots in a randomized incomplete block design consisting of 25 replicates and 13 incomplete blocks per replicate. A total of 246 trees from 13 geographic sub-races were sampled, with 7-13 families per sub-race and 2 trees per family. One sub-race was chosen to represent each of the 13 geographic races of *E. globulus*. Ten fully expanded adult leaves were randomly collected from the mid-canopy on the north side of each tree for cuticular wax extraction and analysis in

April 2012. Leaves were sealed in plastic bags in the field and stored at -20°C until extraction (July-August 2012).

Cuticular wax extraction and analysis was done using a method originally designed for essential oils as this allowed for both oils and waxes to be assessed simultaneously. Cuticular waxes were extracted by immersing 1 gram of wet leaf cut into 6 mm diameter disks from a pool of the 10 leaves in 10 mL of dichloromethane containing 100 mg heptadecane (C17) per litre as an internal standard. Samples were left to extract at room temperature overnight followed by ultrasonication for 30 minutes in a Unisonics FXP-12 M. Extracts were decanted and stored at 4°C. The extraction process was repeated two more times on the same leaf disks, yielding a pooled 30 mL of extract per sample. Surplus leaf material was oven-dried for 7 days at 60°C to determine percentage dry matter.

Extracts were analyzed by gas chromatography-mass spectrometry (GC-MS) using a Varian 3800 GC coupled to a Bruker-300 triple quadrupole MS. Helium was used as the carrier gas, with a pressure program of 9.7 psi to 18.l8 psi at 0.46 psi/min, then to 38 psi at 15psi/min, then to 45 psi at 1.12 psi/min with a final hold time of 12 mins. Waxes were separated with 30 m x 0.25 mm (internal diameter) VF5-ms column, with a 0.25 μm film thickness. One microliter injections were made with an injection port temperature of 280°C and a 3:1 split, 3 minute solvent delay. Initial column temperature was 60°C for 1 min, raised to 210°C at 8°C/min, then raised to 300°C at 12°C/min with a final hold time of 12 mins. Mass spectra was collected from m/z 35 to 650, scanned in 0.2s, plus Selected Ion Monitoring of the following characteristic ions for 30ms per ion; m/z 58, 82, 91, 100, 104,108. Individual compounds were identified bases on an 'in-house' MS database of wax compounds, in tandem with the Kovats' retention index of the analyses. Concentrations of individual compounds are expressed as equivalents of heptadecane, on a milligram per gram of leaf dry matter basis. Overall, nine aliphatic ester, two aliphatic β-diketones and two flavonoid cuticular wax compounds were quantified from these adult leaf samples (Table 4.1), all of which had been previously reported in juvenile leaves (Jones *et al.* 2002; Li *et al.* 1997) and a few in adult leaves of *E. globulus* (Li *et al.* 1997).

Table 4.1. Trial means and quantitative genetic parameters describing the variation in leaf cuticular wax compounds in a range wide study of *Eucalyptus globulus* (Experiment 1).

Cuticular Wax Compound	Code	Mean (mg/gDM)	Sub-race			
			F	P	Q_{st}	H^2
Aliphatic esters						
Benzyl n-tetracosanoate	Benzyl C24	0.085	61.5	**0.000***	**0.39**	**0.40±0.237**
Benzyl n-hexacosanoate	Benzyl C26	0.201	91.7	**0.000***	**0.65***	**0.45±0.266**
Benzyl n-octacosanoate	Benzyl C28	0.127	55.7	**0.000***	**0.28**	**0.47±0.233**
Phenylethyl n-eicosanoate	Phenylethyl C20	0.014	18.9	0.090	0.04	0.45±0.221
Phenylethyl n-docosanoate	Phenylethyl C22	0.022	15.4	0.221	0.03	0.35±0.225
Phenylethyl n-tetracosanoate	Phenylethyl C24	0.066	18.5	0.100	0.05	0.30±0.226
Phenylethyl n-pentacosanoate	Phenylethyl C25	0.008	23.0	0.028*	0.34	0.09±0.227
Phenylethyl n-hexacosanoate	Phenylethyl C26	0.096	21.5	0.043*	0.06	0.38±0.223
Phenylethyl n-octacosanoate	Phenylethyl C28	0.071	29.7	**0.003***	0.08	**0.60±0.218**
Aliphatic β-diketones						
n-Hentriacontane-14, 16-dione	β-diket C31	0.155	74.2	**0.000***	**0.28**	**0.72±0.260**
n-Tritriacontane-16, 18-dione	β-diket C33	4.639	12.3	0.419	0.01	0.47±0.223
Total Wax Yield		5.48	16.4	0.174	0.03	0.38±0.225
Flavonoids						
Desmethyl eucalyptin	Desmethyl	0.089	41.9	**0.000***	0.13	**0.73±0.223**
Eucalyptin	Eucalyptin	0.213	46.8	**0.000***	0.16	**0.62±0.227**

Mixed- model univariate tests of significance of the variation in cuticular wax concentrations between sub-races, the associated quantitative inbreeding coefficient (Q_{st}), and broad-sense heritability estimates (H^2) are shown. Asterisks indicate significance ($p<0.05$), with boldface indicating significance after a Bonferroni adjustment to $p=0.0035$. Q_{st} values significantly different from the reported average F_{st} (0.09) between sub-races for microsatellite markers are shown in boldface, with asterisks indicating Q_{st} values significantly different from the recorded maximum F_{st} (0.201) (see O'Reilly-Wapstra *et al.* 2013b). Statistical significance was tested using a Wald-F test for the fixed sub-race effect.

Phenotypic variation in the composition of all 13 cuticular compounds was summarized with a principal component analysis (PCA) using *prcomp* from the base *stats* package in R version 3.1.2 (R Core Team, 2013). Prior to PCA, compounds were standardized by total yield per sample using *decostand* from the package *vegan* in R. Correlations between cuticular wax compounds were summarized through ordination of the principal components (PCs) with the compound loadings on the components (i.e. correlations between PCs and individual cuticular wax compounds) displayed as vectors.

A linear discriminant analysis maximizing the differences between sub-races was conducted on the raw data of all thirteen cuticular wax compounds using *lda* from the package *MASS* in R. Differences among sub-races were summarized through ordination of sub-race means on the main discriminant axes. The significance of discriminant axes in differentiating between geographic sub-races were tested using *manova* from the base *stats* package in R. Minimum spanning Mahalanobis distances between geographic sub-races were determined based on the Euclidian distances among sub-races in the discriminant space using *spantree* from the package *vegan* in R. Nine climatic variables obtained using home-site data from the natural sub-race populations (sub-races; where seed was originally collected) in ANUCLIM v6.1 (Xu 2011), as well as latitude, longitude, altitude and a drought susceptibility score (Dutkowski and Potts 2012), were fit as vectors into the discriminant space using *envfit* from the package *vegan* in R. This was also done for individual cuticular wax compounds, using sub-race means to provide insight into their influence on sub-race differentiation.

To examine the genetic variation in individual foliar wax compounds, we used the following mixed model fitted with ASReml in R (*asreml-r*) version 3 (Gilmour *et al.* 2009):

$$y = \mu + \text{Sub-race} + \textit{Family(Sub-race)} + \textit{Replicate} + \textit{Residuals}$$

where Sub-race is a fixed effect, and *Family(Sub-race)* and *Replicate* are the random effects corresponding to the variation of families within sub-races and amongst field trial replicates. All compounds, as well as total wax yield (sum of aliphatic esters and diketones), were square root transformed to improve normality and homoscedasticity. Significance of the sub-race fixed effect in the univariate analysis was determined using a Wald-F test. Random effects were tested using a one-tailed log-likelihood ratio test of the full model against a model with the random effect of interest removed.

Variance components for estimating trait heritability and quantitative inbreeding coefficient (Q_{st}) were obtained by fitting sub-race as a random effect in *asreml-r*. Within sub-race narrow-sense heritability estimates (h^2_{op}) and among sub-race quantitative inbreeding coefficients (Q_{st}) were calculated from the variance components obtained from univariate analyses. These analyses

assume base parents from which the families are derived are unrelated and an average coefficient of relationship of 0.4 to account for an assumed selfing rate of 30% in the open-pollinated families. Assumptions, calculations and tests followed those outlined in Dutkowski and Potts (2012) and Hamilton *et al.* (2013). Q_{st} estimates among sub-races were tested to determine if they were greater than the published estimate of the mean microsatellite F_{st} (0.09) as well as the maximum-recorded F_{st} (0.201 in Yeoh *et al*., 2012) using a one-tailed likelihood test. These F_{st} values are from microsatellite studies of similar range-wide populations of *E. globulus* but different individuals. These F_{st} values are relatively stable despite the different studies using different microsatellite loci (e.g. mean Fst values of 0.09 have been reported by both Steane *et al.* 2006; Yeoh *et al.* 2012). If Q_{st} is significantly greater than F_{st}, this is a line of evidence suggesting that diversifying selection has influenced the sub-race divergence (Whitlock 2008).

For individual wax compounds found to vary significantly at the family within sub-race level, pair-wise additive genetic correlations (family within sub-race level correlations) were estimated with a bivariate analysis using *asreml-r* and the same model as above. Inter-trait genetic correlations were tested from zero using a two-tailed likelihood ratio test (Costa e Silva *et al.* 2009). Wax compounds found to vary significantly at the sub-race level were tested for correlations among sub-race least-squares means derived from univariate analyses using Pearson correlations calculated with *cor.test* from the base *stats* package in R. Overall phenotypic correlations for all compounds were tested using bivariate analyses in *asreml-r* with the no model terms fitted. For wax compounds shown to have a significant Q_{st}. Pearson correlations were also used to test for associations of sub-race least-squares means with home-site spatial and climate variables using *cor.test* from the base *stats* package in R. Only climate variables shown to have a significant fit to sub-race variation in the discriminant space were tested.

4.2.2 Experiment 2: QTL and placement of candidate genes

The QTL analyses were conducted on a full-sib outbred F_2 population comprising 112 genotypes, each of which was replicated clonally (two trees per genotype). The population was generated from crossing F1 parents, each derived from crossing unrelated trees from King Island (KI) in Bass Strait and Taranna (T) in south-eastern Tasmania (T7/KI157//KI5/T144; see Freeman *et al*. 2006). The mapping population was planted in a field trial at Woolnorth (40° 52' S, 144° 50' E) in northwest Tasmania in May 1998. The ortet and ramet representing each genotype were assigned to separate replicates at random, and all clonally replicated individuals were used in this study (for full details of the trial design, see Milgate *et al.* (2005b). Juvenile foliage was sampled from 224 trees from the trial in May 2000. Two to four leaves were randomly collected from each tree for cuticular wax extraction and analysis. Leaves were sealed in plastic bags in the field then freeze-dried and stored at room temperature until extraction (August-September 2008).

Wax extractions followed Jones *et al.* (2002) and were analyzed by GC-MS using a Varian CP3800 GC coupled to a Varian 1200L triple quadrupole MS. Helium was used as the carrier gas, with a constant flow rate of 3.5mL/min. Waxes were separated with 30 m x 0.25 mm (internal diameter) VF5-ms column, with a 0.25 μm film thickness. One microliter splitless injections were carried out using a Varian 1079 Programmable Temperature Vaporizing injector with liquid CO_2 coolant. The injection port temperature was set to 30°C for 0.3 minutes, then ramped to 275°C at 200°C/min and held for 5 minutes before cooling to 140°C at 150°C/min. Initial column temperature was 60°C for 1 min, raised to 220°C at 30°C/min, then raised to 310°C at 10°C/min with a final hold time of 8 mins. Mass-spectra were collected from m/z 40 to 550, scanned in 0.22s. Individual compounds were identified based on an 'in-house' MS database of wax compounds, in tandem with Kovats' retention index of the analyses. Concentrations of individual compounds are expressed as equivalents of C22 docosane, on a milligram per gram of leaf dry matter basis. Overall, eleven aliphatic esters, two aliphatic β-diketones, one hydrocarbon, one ketone, one aldehyde and two flavonoid cuticular wax compounds were quantified from these juvenile leaf samples (Table 4.2).

Table 4.2. Putative QTL for cuticular wax compounds in *Eucalyptus globulus* (Experiment 2).

Compound	L.G.[a]	Nearest marker[b]	Map pos (cM)	Peak LOD[c]	% exp[d]	Seg[e]	Mean (mg/gDM)	sd[f]	Clonal repeat.
Aliphatic esters									
Benzyl n-eicosanoate (C20)	6	p03b10	83.3	3.4	13.2	M	0.003	0.004	0.51±0.07
Benzyl n-docosanoate (C22)	1	Emb56	3.5	3.4	10.6	B	0.009	0009	0.47±0.07
	8	599919	1.0	6.1*	19.8	M			
Benzyl n-tetracosanoate (C24)	1	Emb56	3.5	4.1	7.7	B	0.061	0.065	0.63±0.06
	3	567811	109.8	3.3	6.2	F			
	8	599919	1.0	16.0***	38.4	M			
Benzyl n-hexacosanoate (C26)	8	599919	1.0	11.0***	37.1	M	0.093	0.063	0.59±0.06
Benzyl n-octacosanoate (C28)	3	642858	30.2	3.0	8.9	M	0.126	0.070	0.41±0.08
	6	573332	122.2	3.7	10.8	M			
	8	503944	134.6	4.6*	13.8	F			
Phenylethyl n-eicosanoate (C20)	3	642858	30.2	4.9*	14.5	M	0.005	0.005	0.52±0.07
	3	564559	66.3	6.8***	19.3	B			
	5	575337	0.1	4.1	10.6	M			
	6	565210	101.4	3.8	9.6	B			
Phenylethyl n-docosanoate (C22)	3	569486	82.7	10.0***	24.5	B	0.004	0.004	0.49±0.07
	5	p02b02	63.8	4.1	8.9	F			
	7	574475	99.0	3.5	7.5	M			
	9	p10b04	90.6	3.9	8.4	B			
Phenylethyl n-tetracosanoate (C24)	3	p01b01	86.0	6.6**	17.8	B	0.009	0.009	0.65±0.06
	8	599919	1.0	8.1***	23.5	M			
Phenylethyl n-pentacosanoate (C25)	8	599919	1.0	3.1	12.1	M	0.001	0.001	0.25±0.09
Phenylethyl n-hexacosanoate (C26)	3	p01b01	86.0	9.4***	29.5	B	0.012	0.010	0.57±0.07
	9	p10b04	90.6	4.5*	13.0	F			
Phenylethyl n-octacosanoate (C28)	3	p01b01	86.0	6.2*	20.3	B	0.026	0.024	0.72±0.05
	10	562745	4.9	3.1	9.4	-			
Aliphatic β-diketones									
n-Hentriacontane-14, 16-dione (C31)	8	503660	19.5	10.6***	30.8	M	1.10	0.440	0.54±0.07
	11	CRC2	77.9	3.1	7.7	B			
n-Tritriacontane-16, 18-dione (C33)	1	639448	73.0	3.8	10.2	M	10.27	1.96	0.25±0.09
	8	p11b07	35.7	6.2***	17.6	M			
	11	571276	64.2	3.4	9.2	B			

Continued next page…

Compound	L.G.[a]	Nearest marker[b]	Map pos (cM)	Peak LOD[c]	% exp[d]	Seg[e]	Mean (mg/gDM)	sd[f]	Clonal repeat.
Hydrocarbons									
n-Nonacosane	11	562840	54.9	5.5**	20.7	B	0.022	0.077	0.29±0.09
Ketones									
Heptadecanone	1	567923	11.7	5.7*	16.5	M	0.157	0.027	0.32±0.09
	8	566660	15.2	4.0	11.4	M			
	11	571397	63.6	3.5	9.8	B			
Aldehydes									
Hexadecanal	3	571717	15.0	4.5*	13.5	B	0.496	0.130	0.13±0.09
	6	Emb173	89.8	3.2	9.2	F			
	10	570364	6.3	4.3*	12.6	F			
Total wax	1	Emb12	68.1	3.2	7.6		12.571	2.220	0.27±0.09
	3	CSA_1872	4.7	3.2	7.8				
	8	573772	117.8	6.0***	14.9				
	11	571276	64.2	6.4***	16.5				
Flavonoids									
Desmethyl eucalyptin	1	565878	64.4	9.8***	18.2	M	0.415	0.162	0.24±0.09
	2	575243	0.0	3.6	5.8	F			
	3	640146	120.3	9.1***	16.6	B			
	5	p02b02	63.8	5.1**	8.4	-			
	8	562846	23.8	7.1***	12.3	M			
	10	Emb155	101.3	3.5	5.6	B			
	11	575083	75.9	6.0***	10.1	M			
Eucalyptin	1	Emb180	59.5	16.5***	46.2	M	0.385	0.136	0.43±0.08
	5	571218	66.30	3.8	8.1	-			
***Mnesampela privata* (AGM)**	3	642858	30.2	4.3*	12.3	B			
	8	599919	1.0	7.3***	22.7	M			

[a] Linkage group.

[b] Markers names beginning with Emb or CRC are microsatellites, those named p#b# are AFLP, while the remainder are DArT.

[c] Peak LOD score for each QTL. Genome-wide significance is indicated by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The remaining QTL were significant at the suggestive level (chromosome-wide type I error rate < 0.05).

[d] The proportion of total variation explained by each QTL.

[e] Whether the QTL segregated only from the male parent (M), the female parent (F) or from both (B).

[f] sd is the standard deviation of the mean for each compound concentrations.

We used a previously published sex-averaged linkage map of the F2 pedigree for QTL detection (Freeman *et al.* 2006; Hudson *et al.* 2012a). This map includes amplified fragment length polymorphism (AFLP), microsatellite (SSR), Diversity array technology (DArT), and cleaved amplified polymorphic sequence (CAPS) markers. The procedure for DNA extraction and quantification as well as genotyping of AFLP and SSR markers is described in Freeman *et al.* (2006). Genotyping of DArT markers is described in Hudson *et al.* (2012b) and CAPS markers in Freeman *et al.* (2013). Only markers with less than 5% missing data were included in the linkage map. Individual parental maps were initially constructed using an iterative approach (for more detail see Freeman *et al.* 2013). These parental maps were then merged to form a high density (695 markers) sex-averaged map based on biparentally segregating markers. From the sex-averaged map, a subset of 253 evenly spaced markers was chosen (comprising 39 AFLP, 35 SSR, 174 DArT and 5 CAPS) to reduce computational demands during QTL analysis (Van Ooijen 2009). These markers were chosen to maximize map coverage, and provide even representation of markers segregating from each parent (Freeman *et al.* 2013).

The putative eucalypt homologs of well characterized genes implicated in variation in cuticular wax compounds in *Arabidopsis* were also mapped in this study. These homologs were identified by reciprocal BLAST searches of the *E. grandis* (v1.1) and *Arabidopsis* (TAIR10) genomes (http://www.phytozome.net), using all loci from Samuels *et al.* (2008) and Lee and Suh (2013). Putative homologs were accepted only when the original *Arabidopsis* locus was the closest match to the *E. grandis* protein in question. The only exception was a locus from Lokesh *et al.* (2013) which was also included because it was the best hit in the cross validation of CER6 (see Appendix C Table 4.S1). All homologs were then placed on the linkage map based on extrapolation using the position of their closest flanking DArT markers in the *E. grandis* genome sequence (annotated at http://eucgenie.bi.up.ac.za/) using the "neighbors" approach (Cone *et al.* 2002).

Genotype means were used for QTL analysis, hence some of the environmental effects were removed and the power of QTL detection was enhanced. QTL analysis was conducted with MAPQTL 6.0, using the regression approximation to maximum likelihood mapping (Van Ooijen 2009). Clonal repeatability (broad-sense heritability or H^2) was calculated from variance components obtained by fitting genotype as a random effect in a univariate analysis for each

compound in *asreml-r*. Putative QTL were declared at two different levels, significant (genome-wide type I error < 0.05) and suggestive (chromosome-wide type I error rate < 0.05). LOD thresholds for genome-wide and chromosome-wide significance as well as interval mapping and multiple QTL model (MQM) analyses were conducted as described previously (Freeman *et al.* 2008a), except only forward selection of cofactors was used, and MQM mapping used the restricted MQM mapping procedure (see Van Ooijen 2009). QTL segregation was determined by testing whether adjacent markers segregating solely from the male or female parent had a significant effect on the trait in question using Kruskal-Wallis test.

4.3 Results

In this study, two experiments were undertaken to explore the genetic basis of variation in cuticular wax compounds in *E. globulus*. In experiment 1, quantitative genetics analysis of data obtained from a common environment trial was used to identify spatial patterns of genetic variation in cuticular waxes across the geographic range of the species, as well as to test for signals of diversifying selection. Experiment 2 used a clonally replicated F_2 population of *E. globulus* to identify QTL influencing variation in these compounds. We found significant variation in cuticular wax compounds across the geographic range of *E. globulus* with evidence of diversifying selection for four compounds. A total of 52 QTL were identified for these compounds.

4.3.1 Experiment 1

Nine aliphatic esters (three benzyl alkanoates and six phenethyl alkanoates), two aliphatic β-diketones and two flavonoid compounds were quantified in the adult leaf samples (Table 4.1). Phenotypic variation in these compounds was continuous and multidimensional in the principal component ordination, but variation in many compounds was highly inter-correlated (Fig. 4.1a & 4.1b). Strong correlations were particularly evident within the benzyls (C24-C28) and the phenylethyls (C20-C28), with compounds with different carbon chain lengths often showing similar correlations (Table 4.3). Prior to Bonferroni adjustment, nine of the thirteen compounds showed significant variation at the sub-race level (Table 4.1). However, there is little evidence of significant within sub-race genetic variation between families, most likely due to the low number of replicates per family. The differentiation of the sub-races could be mainly explained by independent latitudinal and longitudinal gradients. The greatest difference was due to variation

between the mainland and Tasmanian sub-races (Fig. 4.2a), which was mainly associated with increasing maximum temperatures (Fig. 4.2c). The Western Tasmania sub-race was intermediate between the mainland and the other Tasmanian sub-races in the minimum spanning tree, reflecting a well-recognized western pattern of historic migration for the species (Freeman *et al.* 2001). The latitudinal separation is predominantly due to the more northern, mainland sub-races having lower levels of phenylethyl C28, the minor β-diket C31 and desmethyl (Fig. 4.2b; see Appendix C Fig. 4.S1; All compound abbreviations used throughout are listed in Table 4.1). A similar pattern of longitudinal differentiation was evident on the mainland and the island of Tasmania (Fig. 4.2a). This was mainly due to an easterly increase in benzyl alkanoates (see Appendix C Fig. 4.S1), and was associated with increasing temperature seasonality, decreasing precipitation seasonality and increasing drought resistance of the *E. globulus* sub-races (Fig. 4.2c).

Four of the cuticular wax compounds involved in both longitudinal (benzyls C24, C26 & C28) and latitudinal (β-diket C31) differentiation of sub-races showed evidence of diversifying selection. First, their Q_{st} values were significantly greater than the reported average microsatellite F_{st} values (Table 4.1), and the Q_{st} of one (benzyl C26; see Appendix C Fig. 4.S1) was significantly greater than the maximum microsatellite F_{st}. Second, there was clear environmental variation associated with the sub-race variation, with two of these benzyls decreasing with increasing precipitation seasonality and β-diket C31 increasing with colder home-site temperate (Fig. 4.3).

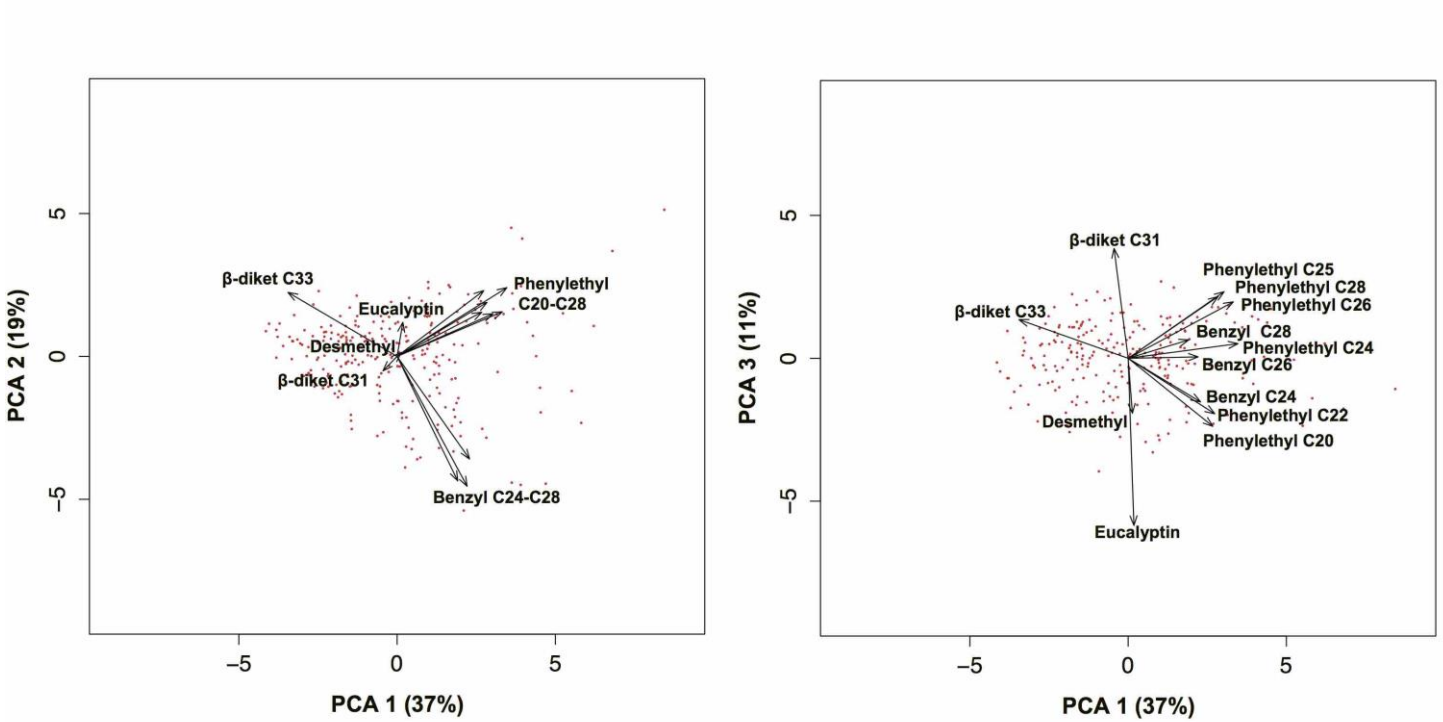

Fig. 4.1. Visual representation of cuticular wax composition in a range wide study of *Eucalyptus globulus* through ordination of the first three principal components from the PCA of all 13 cuticular wax compounds in experiment 1. Together PC1-3 explained 67% of the variation. Red dots represent individual trees in the trial. Correlations of individual compounds with the principal components are displayed as vectors.

Table 4.3. Matrix of phenotypic (pheno) and sub-race (sub) correlations between cuticular wax compounds in a range-wide study of *Eucalyptus globulus* (Experiment 1).

		Benzyl C24	Benzyl C26	Benzyl C28	Phenyl-ethyl C20	Phenyl-ethyl C22	Phenyl-ethyl C24	Phenyl-ethyl C25	Phenyl-ethyl C26	Phenyl-ethyl C28	β-diket C31	β-diket C33	Desmethyl
Benzyl C26	pheno	**0.80***											
	sub	**0.94***											
Benzyl C28	pheno	**0.68***	**0.87***										
	sub	0.80*	**0.90***										
Phenylethyl C20	pheno	**0.42***	**0.28***	**0.23***									
	sub	NA	NA	NA									
Phenylethyl C22	pheno	**0.52***	**0.32***	**0.27***	**0.78***								
	sub	NA	NA	NA	NA								
Phenylethyl C24	pheno	**0.54***	**0.48***	**0.44***	**0.65***	**0.85***							
	sub	NA	NA	NA	NA	NA							
Phenylethyl C25	pheno	**0.45***	**0.45***	**0.39***	**0.40***	**0.54***	**0.75***						
	sub	0.33	0.37	0.51	NA	NA	NA						
Phenylethyl C26	pheno	**0.43***	**0.59***	**0.52***	**0.46***	**0.58***	**0.86***	**0.77***					
	sub	0.47	0.59*	0.72*	NA	NA	NA	**0.84***					
Phenylethyl C28	pheno	**0.32***	**0.47***	**0.57***	**0.38***	**0.52***	**0.80***	**0.70***	**0.90***				
	sub	0.11	0.22	0.55	NA	NA	NA	0.77*	**0.83***				
β-diket C31	pheno	**0.29***	**0.39***	**0.42***	**0.33***	**0.28***	**0.40***	**0.31***	**0.41***	**0.42***			
	sub	0.13	0.29	0.54	NA	NA	NA	0.40	0.60*	0.71*			
β-diket C33	pheno	**0.35***	**0.43***	**0.43***	**0.36***	**0.41***	**0.52***	**0.36***	**0.51***	**0.49***	**0.77***		
	sub	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		
Desmethyl	pheno	0.10	**0.23***	**0.30***	0.16	0.20	**0.30***	0.14	**0.35***	**0.39***	**0.47***	**0.55***	
	sub	0.16	0.30	0.56*	NA	NA	NA	0.49	0.69*	**0.82***	**0.86***	NA	
Eucalyptin	pheno	0.03	0.10	0.12	0.22	0.16	0.18	0.08	0.20	0.20	0.16	**0.43***	**0.38***
	sub	-0.56*	-0.70*	-0.72*	NA	NA	NA	-0.11	-0.40	-0.29	-0.64*	NA	-0.52

NA = no correlation analysis was undertaken due to lack of significance from the univariate analyses.
* significant correlation prior to Bonferroni adjustment of the p-value.
Boldface indicates significant correlation post Bonferroni adjustment of the p-value (phenotypic p=0.0006; sub-race p=0.0014).

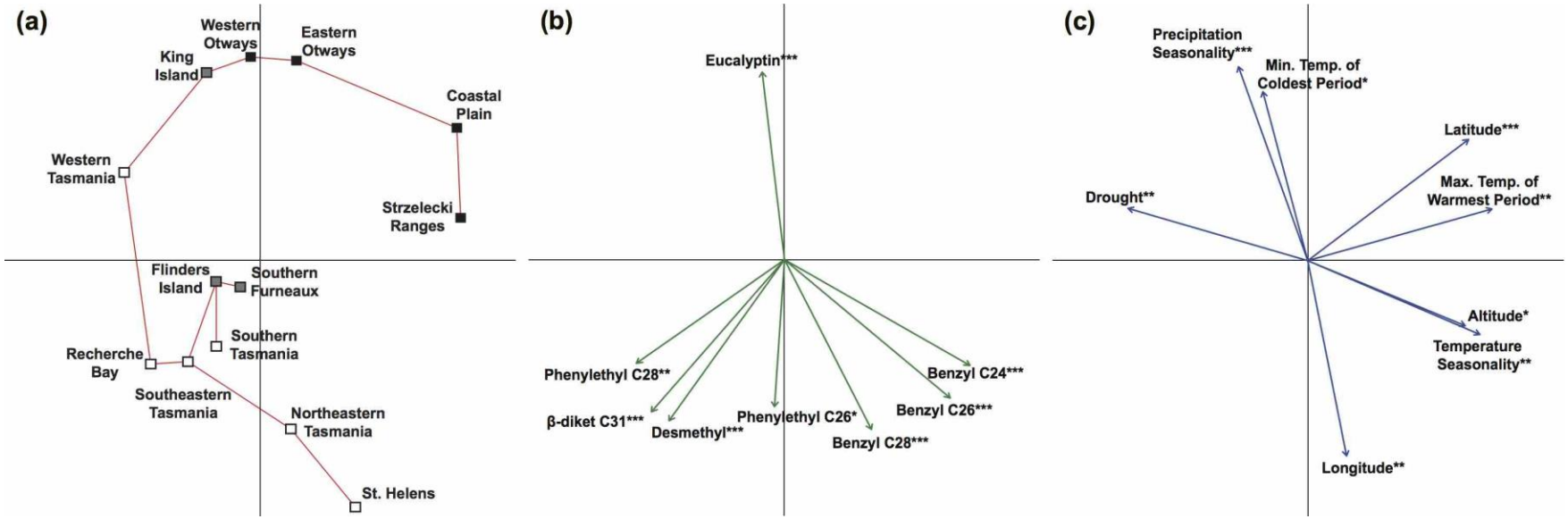

Fig. 4.2. a) Ordination plot summarizing the variation in all thirteen cuticular wax compounds between geographic sub-races of *Eucalyptus globulus* produced from a linear discriminant analysis (LDA). Separation along CV1 (vertical; F=25.2, p<0.001) and CV2 (horizontal; F=10.9, p<0.001) accounted for 47% and 22% of the variation between sub-races in the LDA, respectively. Lines connecting sub-races are the minimum spanning Mahalanobis distances between sub-races. Black squares represent mainland sub-races, grey squares represent island sub-races between the mainland and Tasmania, and white squares represent Tasmanian sub-races. b) The significant fitted vectors for individual cuticular wax compounds; and c) the significant fitted bioclimatic vectors. Asterisks indicate the degree of significance. Significance of vectors are indicated by * P < 0.05, ** P < 0.01, *** P < 0.001.

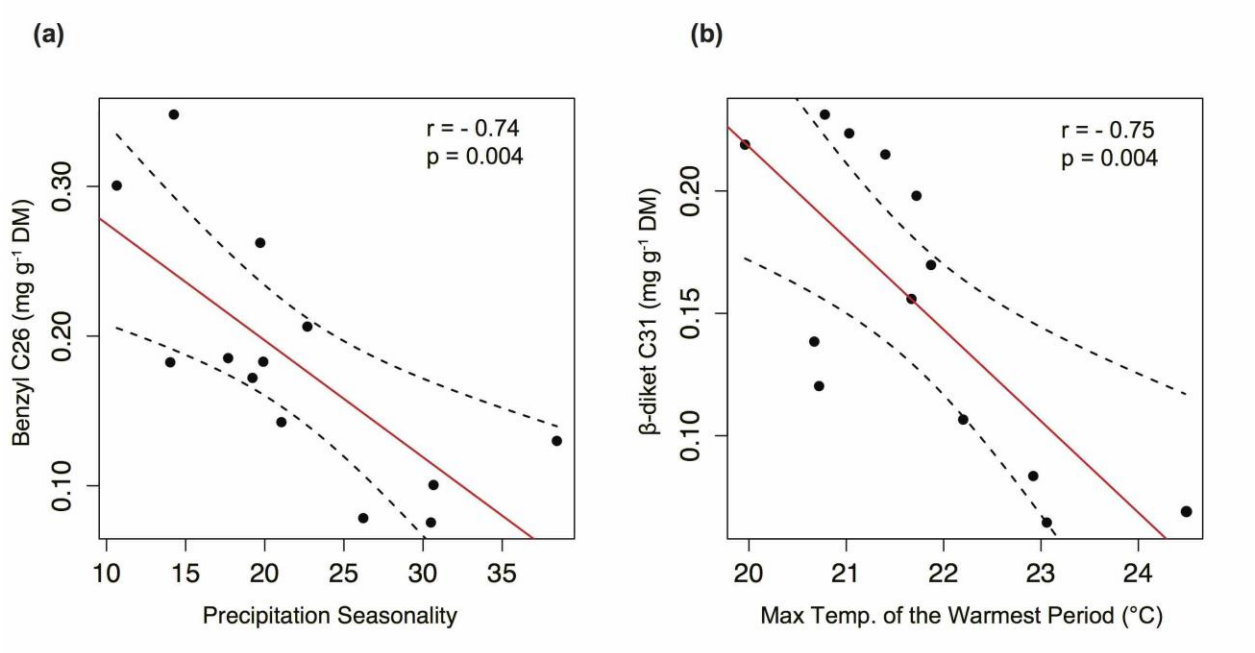

Fig. 4.3. Scatter plot showing the relationship between, a) benzyl alkanoate C26 and precipitation seasonality, and b) β-diketone C31 and the maximum temperature of the warmest period with 95% confidence interval (dashed line) of the regression (red line) in *Eucalyptus globulus*. The Pearson correlation coefficient and their respective significance are displayed in the top right corner of each plot. Values for cuticular wax compounds represent the sub-race least-squares means from a linear mixed-model analysis.

4.3.2 Experiment 2

Eleven aliphatic esters (five benzyls and six phenylethyls), two aliphatic ß-diketones, one hydrocarbon, one ketone, one aldehyde and two flavonoid compounds were quantified in the juvenile leaf samples (Table 4.2). All waxes observed in adult leaves were also found in juvenile leaves and the principal wax was the same in both leaf types (ß-diket C33; 82% of total wax in juvenile versus 85% in adult leaves). The second most common wax in adult leaves (benzyl C26) was the third most common in the juvenile. The ratio of flavonoids to waxes was also very similar between leaf types (0.06 in juvenile versus 0.05 in adult). This apparent stability is consistent with the strong correlation between wax composition of juvenile and adult foliage at the species level in eucalypts (Li *et al.* 1997). For example, the species-level correlations between paired adult and juvenile samples from the same native forests averaged 0.80 (sd=0.2) across the 46 wax compounds they studied. Within the family studied here, clonal repeatability was moderately high (avg.=0.44; Table 4.2), indicating the importance of genetic factors in controlling the variation in these compounds.

Fifty-two QTL were identified at the chromosome-wide significance level (Type I error rate < 0.05; Table 4.2). One to seven QTL were identified for each compound, with each QTL explaining an estimated 5.6-46.2% of the variation between genotype means. Genomic regions impacting the 18 compounds and total wax yield (sum of aliphatic esters, diketones, ketones, hydrocarbons and aldehydes) were spread throughout most of the genome, occurring on 10 of the 11 chromosomes. However, consistent with strong positive correlations between compounds in Experiment 1 (sub-race and/or phenotypic; Table 4.3), QTL for many wax compounds were found to co-locate (ie. their 95% confidence intervals overlapped) and many of these shared the same peak (Fig. 4.4). Of the 24 genes involved in biosynthesis, secretion or regulation of biosynthesis of wax compounds in *Arabidopsis* (as listed in Lee and Suh 2013; Samuels *et al.* 2008), putative orthologs for 18, including the *KCS11* loci from Lokesh *et al.* (2013) described in the methods, could be unambiguously identified in the *E. grandis* genome and were positioned on the linkage map (see Appendix C Table 4.S1; Fig. 4.4). Seven of these candidate genes occurred within 3cM of the peak location of one or more QTL (*CER3*, *FATB*, *SHN1*, *CER10*, *KCS11*, *WBC11*, and *WAR4*). We considered these good positional candidates for the QTL in question, because the locus (or loci) underlying QTL effects are often very close to the QTL peak (Price 2006). We searched the region

of the *E. grandis* genome 1 Mbp on either side of the QTL peaks to verify none of the annotated genes had gene ontology related to very long chain fatty acid biosynthesis or cuticular wax variation, providing additional support for these loci as positional candidates.

For the four compounds exhibiting signals of diversifying selection (benzyls C24, C26, C28 and β-diket C31; Table 4.1), nine QTL were identified on five chromosomes (Table 4.2 and Figure 4.4). Notable was the co-location of highly significant QTL for benzyls C24 and C26 on chromosome 8 with the candidate gene KCS11. QTL for several other wax compounds (phenylethyls C24 and C25 and benzyl C22), as well as damage by the Autumn Gum Moth larvae (AGM; using data from Jones *et al.* 2002) also co-locate at this point. In all six cases, these QTL peaked at exactly the same location, were inherited solely from the male parent with genotype means consistent with the direction of the correlations between these traits (see Appendix C Table 4.S2). Of the other compounds suggestive of diversifying selection, two other co-locations between QTL and candidate genes were noteworthy. First, was the co-location of WAR4 with the strongest QTL for benzyl C28 on chromosome 8. Second, was the co-location of a wax gene WBC11 and a QTL peak for the stronger of the two QTL for β-diket C31 on chromosome 8. While QTL peaks for β-diket C31 and β-diket C33 did not coincide, their degree of independence is unclear as all QTL for β-diket C31 overlap the 95% confidence interval of QTL for β-diket C33. Furthermore, the QTL for β-diket C31 and C33 on chromosome 8 have the same segregation pattern, providing further evidence they may be influenced by the same gene.

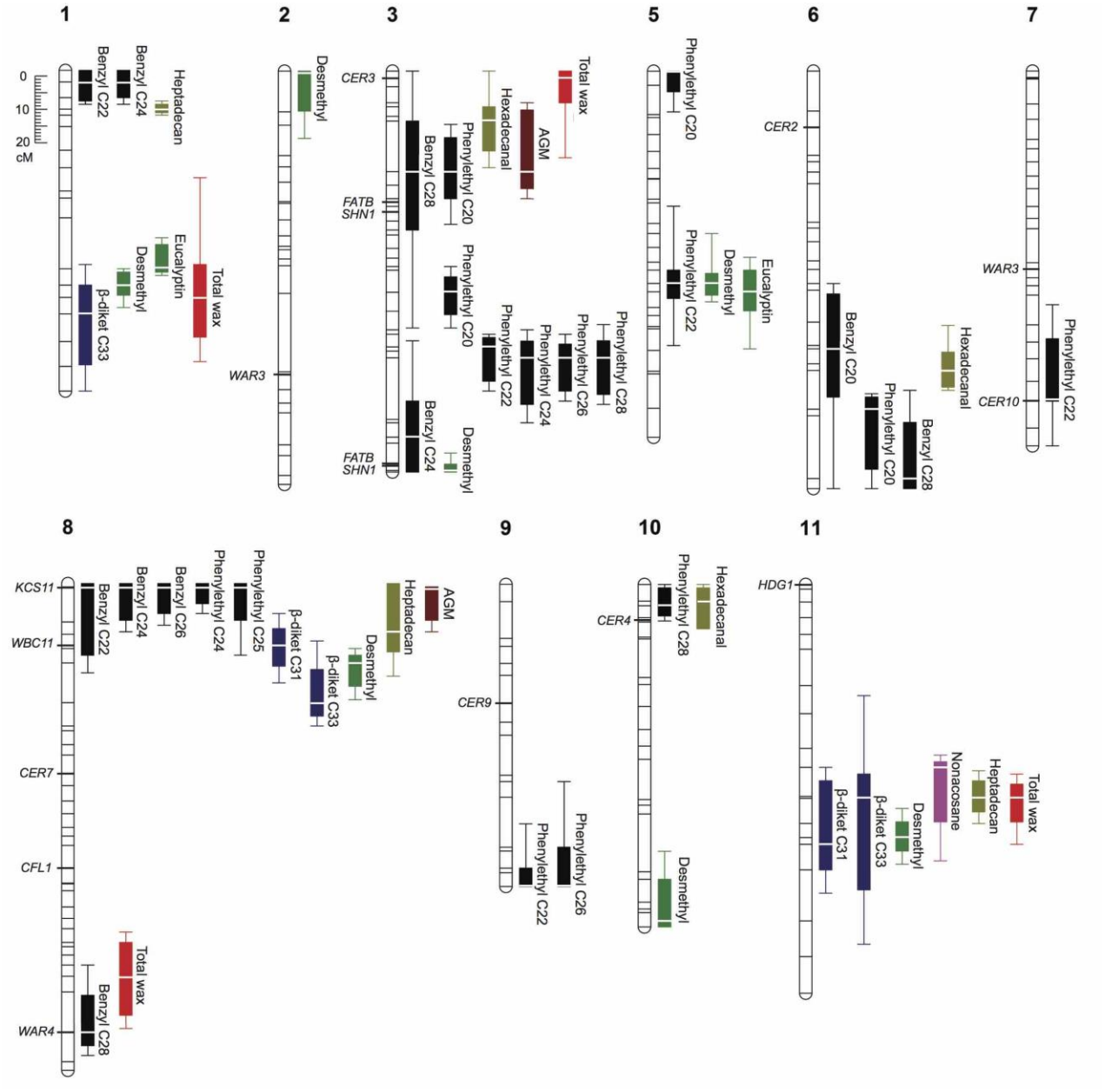

Fig. 4.4. Putative QTL locations for the 18 cuticular wax compounds of juvenile foliage samples in *Eucalyptus globulus*, total wax yield and damage caused by autumn gum moth (AGM) in 11 linkage groups through interval and MGM mapping. Linkage groups are described in Freeman *et al.* (2006). Candidate gene locations are displayed to the left of linkage groups (see Appendix C Table S3). White bars indicate the peak LOD. For each QTL, solid bars and lines represent one and two LOD support intervals, respectively. The two-LOD support interval corresponds approximately to a 95% confidence interval (Lander and Botstein 1989). Colors represent the differing biosynthetic pathways, as well as a different color for total wax yield.

4.4 Discussion

A large number of QTL were detected, reflecting the generally medium to high broad-sense heritability and clonal repeatability of the cuticular compounds. These QTL included several of relatively large effect, many of which affected multiple compounds produced from the same biochemical pathway. While most compounds studied were wax esters derived from the primary alcohol pathway, we found QTL affecting compounds associated with the three distinct biosynthetic pathways for cuticular waxes (Le Provost *et al.* 2013), as well as the flavonoid pathway (phenylpropanoid pathway; Falcone Ferreyra *et al.* 2012). Most QTL co-location involved compounds from the primary alcohol pathway, consistent with these QTL representing polymorphism in genes acting within this pathway (Samuels *et al.* 2008). In some cases this co-location specificity was for QTL affecting benzyl and phenylethyl alkanoate compounds within the primary alcohol pathway, which indicates polymorphisms in genes operating later in this biosynthetic pathway. For compounds that are products of each biosynthetic pathway, we also found signals of selection and QTL, which co-located with candidate genes. Further annotation of the eucalypt homologs of genes implicated in cuticular wax variation will no doubt yield additional positional candidates. Nonetheless our preliminary annotation identified seven candidate genes that occurred <3cM from the peak location of one or more QTL. These include genes involved in cuticular wax biosynthesis (*CER4, CER10, FATB, KCS11, WBC11*), secretion (*WBC11, CER3*), and regulation of wax biosynthesis (*SHN1* [transcriptional regulation], *WAR4* [mRNA stability]).

Four independent QTL were identified for total wax yield with, each on a different chromosome, were found for total wax yield. The most significant QTL for total wax coincided with that for the major wax component, β–diket C33 on chromosome 11. β–diketones are not present in Arabidopsis and rice and thus their genes are less well known (Zhang *et al.* 2013), however, but they are the dominant wax compounds in many eucalypts (Li *et al.* 1997). Also notable was the QTL for total wax on chromosome 3, which was independent of QTL for individual compounds and co-located with the candidate gene *CER3*. *CER3* is involved in the regulation of cuticular wax deposition on inflorescence stems of Arabidopsis (Lam *et al.* 2012; Lee and Suh 2013). This gene affects the relative levels of compounds in the alkane pathway (e.g. Samuels *et al.* 2008), and is up-regulated following drought stress in *Pinus pinaster* (Le Provost *et al.* 2013).

Arabidopsis has three *SHN* homologs; of these *Eucalyptus* has two putative homologs of *SHN1* and lacks homologs of the other two (Borisjuk *et al.* 2014; Marques *et al.* 2012). In *Arabidopsis*, the well-studied *WIN1/SHN1* is a AP2/EREBP-type transcription factor which was originally defined as an activator of cuticular wax biosynthesis, but later reports suggests it directly regulates cutin biosynthesis and indirectly affects cuticular wax production (Lee and Suh 2013; Li-Beisson *et al.* 2010). Nevertheless, overexpression of *SHN1* has been shown to result in up-regulation of genes such as *CER2*, and affect various alkanes, aldehydes and mid-chain hydroxylated fatty acids (Borisjuk *et al.* 2014). It also influences diverse traits including cuticular wax deposition (Kannangara *et al.* 2007); epidermal architecture (Shi *et al.* 2013); drought tolerance (Al-Abdallat *et al.* 2014; Yang *et al.* 2011); and disease response (Sela *et al.* 2013). Both of the eucalypt *SHN1* putative homologs are on chromosome 3, and these genes do not co-locate with any observed QTL for total wax or wax compounds. Rather *SHN1* co-locates with a highly significant QTL for desmethyl eucalyptin, a flavonoid believed to have anti-microbial activity (Takahashi *et al.* 2004). The association of *SHN1* with desmethyl eucalyptin does not appear to have been reported previously and may be a novel effect of this transcription factor.

Of the genes co-locating with QTL for compounds exhibiting signals of diversifying selection, *KCS11* is the most noteworthy. *KCS* genes encode one of four enzymes (3-ketoacyl-coA synthase) involved in a series of consecutive reactions that result in the elongation of C16 and C18 fatty acids into the very-long-chain fatty acids which are subsequently modified into the major wax compounds (Borisjuk *et al.* 2014). This elongation is believed to be a rate-limiting step in wax biosynthesis (Seo and Park 2011). Of the 21 *KCS* loci annotated in *Arabidopsis*, a subset have been implicated in cuticle formation (*CER6, CUT1, KCS1, KCS2, DAISY, KCS20* and *FDH*; Lokesh *et al.* 2013). A putative eucalypt homolog of *KCS11* was co-located with multiple wax ester QTL on chromosome 8, affecting both benzyl and phenylethyl alkanoates, consistent with this locus being rate limiting and early acting in the biosynthesis of wax compounds.

While there was no significant sub-race differentiation in the dominant wax component β-diket C33, the minor compound β-diket C31 showed signals of diversifying selection. The QTL for β-diket C31 co-located with the QTL for heptadecanone on chromosome 8 and both segregated solely from the male parent and had genotype mean differences consistent with the patterns of co-

variation observed in the quantitative study. Both these QTL overlap with *WBC11*. *WBC11* is an, which is an ATP binding cassette (ABC) transporter and part of an evolutionary conserved family of transporter genes operating in diverse organism including plants, humans and Drosophila (Samuels *et al.* 2008). In Arabidopsis *WBC11* is expressed in the plasma membrane of stem epidermal cells (Samuels *et al.* 2008), and mutants have decreased wax loads and alkane concentrations. Heptadecanone is a ketone rather than a diketone, and as these compounds are in different pathways, any pleiotropic effect of *WBC11* on these two compounds could be due to co-transportation rather than polymorphisms affecting their biosynthesis.

In line with the distinct biochemical pathways involved in the biosynthesis of the cuticular compounds studied, the diverse and often discrete QTL detected argue that the patterns of phenotypic and sub-race differentiation observed in the quantitative study have a complex genetic origin. The major directions of phenotypic variation in the wild were due to independent variation in different compound classes. Most notable was the independent variation of the benzyl and phenylethyl alkanoates, with correlated variation of compounds of the same class but different chain lengths. This was also reflected at the sub-race level, where independent directions of differentiation involved different compound classes.

The overall sub-race differentiation in the wax compounds appears to reflect historical gene flow. It is broadly consistent with hypothesized seed-mediated migration routes as revealed through maternally inherited chloroplast DNA (Freeman *et al.* 2001). These chloroplast data revealed a western link between mainland and island populations of *E. globulus* (Western Otways, King Island and Western Tasmania sub-races), and an eastern disjunction between Northeastern Tasmania and the Furneaux islands (Flinders Island/Southern Furneaux). It also revealed a link between western and eastern Tasmanian sub-races through the southern sub-races. The chemical affinities are also generally consistent with the patterns of nuclear marker divergence, which separate mainland Australia from the island of Tasmania as the first order of structure, as well as eastern and western sub-races on both mainland Australia and on Tasmania (Jones *et al.* 2013; Steane *et al.* 2006; Yeoh *et al.* 2012). The latitudinal variation across the range of *E. globulus* means that it is difficult to unravel the effects of geographic (e.g. migration routes) and environmental gradients (Freeman *et al.* 2001; Hamilton *et al.* 2013; Yeoh *et al.* 2012).

Nevertheless, some specific compounds exhibit greater sub-race divergence than would be expected through drift alone, which when coupled with demonstrable environment correlates, is suggestive of diversifying selection (Whitlock 2008). Diversifying selection has long-been thought to shape population genetic differentiation in foliar wax phenotypes in eucalypts (Barber and Jackson 1957; Potts and Jackson 1986). This divergence is reflected in parallel clines from green to glaucous wax phenotypes within different species with increasing altitude and/or insolation (Barber, 1955) (Barber 1955). This change in wax phenotypes is associated with functional differences in foliar surface water repellency (Neinhuis and Barthlott 1997; Silva Fernandes 1965) and reflectance (Close *et al.* 2007). It is also associated with changes in the relative proportion of n-alkanes to β-diketone compounds in the foliar wax (Li *et al.* 1997; Wirthensohn *et al.* 1999), as well as differences in hydrocarbon chain lengths (Hall *et al.* 1965).

In the present study, sub-race divergence involved independent differentiation in compounds from three biosynthetic pathways (the primary alcohol pathway, the ß-ketoacyl pathway and the phenylpropanoid pathway), with evidence that compounds in two of these pathways are under independent selection. For example, β-diket C31 increased with decreasing home-site temperature. The other compounds were three benzyl alkanoates of which most divergence was found in C26 and C28, which were both negatively correlated with precipitation seasonality. While the genetic control in the QTL family may not necessarily reflect sub-race variability (the variation in the QTL family may originate from within or between sub-race variation), it is possible that divergence in these two benzyl alkanoates involves independent loci. *KCS11* and *WAR4* are currently our best candidates for such loci. Variation in many of the wax genes has been functionally linked to drought resistance in crop plants and Arabidopsis (Al-Abdallat *et al.* 2014; Seo and Park 2011). While both mainland and island sub-races of *E. globulus* showed overall differentiation in cuticular wax compounds which correlated with their susceptibility to drought in field trials (Dutkowski and Potts 2012), correlations with these individual benzyl alkanoates were not significant. Indeed, the divergence observed may be driven by both abiotic and/or biotic factors. Benzyl and phenylethyl alkanoates appear to be bioactive (particularly benzyl C24), with increased concentrations associated with reduced susceptibility to a major insect pest of *E. globulus* juvenile leaves (the autumn gum moth or AGM; *Mnesampela privata* - Jones *et al.* 2002; Rapley *et al.* 2004b). This association is reflected in the genotype means at the co-located QTL for AGM damage, benzyls

C22, C24 and C26 and phenyls C24 and C25 on chromosome 8, all possibly reflecting the pleiotropic effect of the underlying putative homolog of *KCS11*.

In conclusion, our study of range-wide population and specific family variability highlight the genetic complexity underlying the phenotypic divergence of *E. globulus*. In the mapping family, we showed that many loci distributed throughout the genome influence cuticular chemistry. The positional candidate genes are functionally diverse, ranging from loci involved in gene regulation, biosynthesis, transport, and secretion. Nevertheless, the co-location of most QTL appears to be due to pleiotropy, as co-location is most common for compounds in the same biosynthetic pathway or class. Such pleiotropy can explain the parallel patterns of phenotypic variation observed for many related compounds across the geographic range of *E. globulus*. However, between different groups of related compounds, independent latitudinal and longitudinal differentiation amongst sub-races is consistent with independent divergence in loci affecting compounds from different biosynthetic pathways.

4.5 Acknowledgements

We thank Carmen Whiteley, Paul Tilyard and Justin Bloomfield for collection of the samples involved in the quantitative study, Valérie Hecht for assistance with locating eucalypt homologs of genes from *Arabidopsis*, Forestry Tasmania and Forico Pty Limited for access to the trials. This study is part of a PhD undertaken by Benjamin Gosney, supported by an International Postgraduate Scholarship provided by the University of Tasmania and funded by the Australian Government. Data collection was funded by Australian Research Council Discovery grants (DP140102552 and DP0773686) and a Linkage grant (LP120200380 in partnership with Greening Australia).

Chapter 5: The extended community-level effects of genetic variation in foliar wax chemistry in the forest tree, *Eucalyptus globulus*

Abstract

Genetic variation in foundation trees can influence dependent communities, but little is known about the mechanisms driving these extended genetic effects. We studied the potential chemical drivers of genetic variation in the dependent foliar community of the focal tree *Eucalyptus globulus*. We focus on the role of cuticular waxes and compare the effects to that of the terpenes, a well-studied group of secondary compounds known to be bioactive in eucalypts. The canopy community was quantified based on the abundance of thirty-nine distinctive arthropod and fungal symptoms on foliar samples collected from canopies of 246 progeny from 13 *E. globulus* sub-races grown in a common garden trial. Cuticular waxes and foliar terpenes were quantified using gas chromatography - mass spectrometry (GC-MC). A total of 4 of the 13 quantified waxes and 7 of the 16 quantified terpenes were significantly associated with the dependent foliar community. Variation in waxes explained 22.9% of the community variation among sub-races, which was equivalent to that explained by terpenes. In combination, waxes and terpenes explained 34% of the genetic variation among sub-races. Only a small proportion of wax and terpene compounds showing statistically significant differences between sub-races were implicated in community level effects. The few significant waxes have previously shown evidence of divergent selection in *E. globulus,* which signals that adaptive variation in phenotypic traits may have extended effects. While highlighting the role of the understudied cuticular waxes, this study demonstrates the complexity of factors likely to lead to extended genetic effects in foundation trees.

5.1 Introduction

Community genetic studies have shown extended effects of genetic variation within focal plant species, including influences on dependent fungal, arthropod and vertebrate communities (Hersch-Green *et al.* 2011; Whitham *et al.* 2006; Whitham *et al.* 2012). These extended genetic effects can influence multiple facets of the dependent community, from the abundance of individual

organisms, to overall community richness and composition (Gosney *et al.* 2014; Maddox and Root 1987; Wimp *et al.* 2005). In the case of foundation species such as forest trees, focal tree genetics has been shown to influence dependent communities above- (Barton *et al.* 2015; Gosney *et al.* 2014; Lau *et al.* 2016) and below-ground (Gehring *et al.* 2014; Velmala *et al.* 2013); as well as extend beyond the living tree (e.g. litter – after-life effects; (Compson *et al.* 2015; Compson *et al.* 2016; Korkama-Rajala *et al.* 2007) and to higher trophic levels (Abdala-Roberts and Mooney 2014; Smith *et al.* 2011). These extended effects of focal tree genetics can result from different scales of genetic variation, ranging from interspecific hybridization (Dungey *et al.* 2000; Pérez-López *et al.* 2016), provenance (Barbour *et al.* 2009c; Gosney *et al.* 2014; Sinclair *et al.* 2015) and family variation within species (Axelsson *et al.* 2015), to specific gene (Zinkgraf *et al.* 2016) or QTL (DeWoody *et al.* 2013) effects.

Photochemistry has long been implicated as a key mechanism driving these community genetic effects (Macias *et al.* 2007; Maldonado-Lopez *et al.* 2015; Whitham *et al.* 2006; Wimp *et al.* 2007). At the level of individual organisms, plant secondary metabolites can influence insect and mammalian herbivores, as well as the fungal pathogens of many plant species (Bennett and Wallsgrove 1994; Edwards *et al.* 1990; Mithöfer and Maffei 2016; Rosenthal and Berenbaum 2012). For example, levels of insect herbivory and mammalian browsing have been shown to decrease with increasing concentrations of the foliar terpene, cineole (Bustos-Segura *et al.* 2015; Stone and Bacon 1994; Wiggins *et al.* 2003). At the community level, plant secondary metabolites have been shown to be associated with variation in community abundance, richness and composition. For example, the abundance of leaf miners on *Quercus castanea* has shown a negative association with a number of secondary metabolites (Maldonado-Lopez *et al.* 2015). In numerous *Quercus* tree species, canopy arthropod species abundance and richness decreased with increasing levels of condensed tannins (Cornelissen and Stiling 2006; Forkner *et al.* 2004; Yarnes *et al.* 2008). Similarly, concentrations of condensed tannins in both *Populus spp*. (Whitham *et al.* 2006) and *E. globulus* (Barbour *et al.* 2009c) have been shown to correlate significantly with variation in the canopy community composition. While only investigated in relation to the canopy community in *E. globulus*, this correlated relationship with condensed tannins extends to the endophyte and aquatic communities in *Populus spp*., indicating the importance of such compounds. While the defensive nature and community genetic effects of secondary metabolites

such as phenols and terpenes has been well-studied, far less attention has been given to other important plant compounds, such as cuticular waxes.

Cuticular waxes are primarily derived from very-long-chain (C20-C34) fatty acids, crystalline in structure and can be either intracuticular or epicuticular (Baker 1982; Kunst and Samuels 2003; Post-Beittenmiller 1996; Samuels *et al.* 2008). They are the primary barrier of a plant to its environment, providing protection from water loss, ultraviolet rays, insect herbivores and pathogens (Riederer and Schreiber 1995; Tucker *et al.* 2010; Yeats and Rose 2013). Despite their importance few studies have examined the extended genetic effects of leaf cuticular waxes on dependent communities. At the level of individual organisms, cuticular waxes have been shown to directly affect insect feeding preference, oviposition choice, and the growth of fungi (Eigenbrode and Espelie 1995; Jarrold *et al.* 2007; Steinbauer *et al.* 2004). For example, waxy leaf (glossy) phenotypes of the crop *Brassicaceae* were found to be less susceptible to feeding by the flea beetle, *Phyllotreta cruciferae*, compared to that of the non-waxy (glossy) phenotypes (Bodnaryk 1992). Cuticular waxes can affect dependent organisms through structural and chemical mechanisms. For example, the eucalypt herbivore, *Paropsis charybdis*, cannot adhere to wax covered leaves of the juvenile stage of its eucalypt host (Edwards 1982). Such effects of waxes may also be indirect as seen in *Brassica oleracea* where the increasing density of leaf wax was found to decrease the ability of key herbivore predator (*Hippodamia convergens*) to adhere to the leaf surface (Eigenbrode 2004). Specific wax compounds themselves may have positive or negative associations with specific organisms (ie. attract some fungi but inhibit others (Reisige *et al.* 2006; Uppalapati *et al.* 2012). While cuticular wax compounds have been found to correlate to host susceptability to specific herbivores or pathogens, as far as we are aware no studies have looked at their broader role in shaping community genetic variation, nor determined their relative importance compared to that of well-known groups of defensive compounds.

In the case of the genus *Eucalyptus*, wax compounds vary between species (Li *et al.* 1997), as well as genetically within species (Gosney *et al.* 2016). Variation in the levels of cuticular wax compounds is often associated with visually detectable wax phenotypes. Green (e.g. *E. ovata*) and glaucous (e.g. *E. globulus*) foliage differences among species is associated with the relative amounts of β-diketones, which are the primary components of the cuticular wax layer in all

eucalypt species (Li *et al.* 1997). Even within foliage of the same wax phenotype, there may be considerable variation in the profile of cuticular wax compounds. In the well-studied *Eucalyptus globulus*, several regions of the genome affect the concentration of cuticular waxes, significant differences in wax profiles have been observed among sub-races in a common environment trial, and three wax compounds exhibit signals of divergent selection (Gosney *et al.* 2016). The primary differentiation in wax profiles within *E. globulus* was associated with independent latitudinal and longitudinal gradients between sub-races, with a southerly increase in a phenylethyl alkanoate, a β-diketone and a flavonoid, and an easterly increase in all benzyl alkanoates.

The present study focuses on understanding the extended genetic effects of the variation in wax chemistry within *E. globulus*. A previous community genetic study of *E. globulus* showed sub-race effects on the dependent arthropod and fungal community which were associated with variation in plant secondary metabolites (tannins and macrocarpal G), and an overall foliar physiochemical profile through near-infrared spectroscopy (NIR) (Barbour *et al.* 2009c). While cuticular waxes were not assayed, they may be involved in the community response to the overall physiochemical profile. With significant genetic based sub-races differences in wax chemistry (Gosney *et al.* 2016), coupled with reported bio-activity of specific wax compounds (phenylethyl and benzyl alkanoates) (Jones *et al.* 2002), we hypothesize that the sub-race variation in wax profiles may explain a significant component of the extended genetic variation in the arthropod and fungal communities observed in canopies of *E. globulus*. As a control for assessing the relative importance of genetic variation in wax chemistry on these canopy communities, we compared the community-level effects of the wax chemicals to that of the terpenes. The terpenes are a well-studied group of secondary metabolites in eucalypts, with documented links to foliar herbivory (Stone and Bacon 1994) and microbial activity (Batish *et al.* 2008; Oyedeji *et al.* 1999).

5.2 Methods and materials

5.2.1 Field trial & sampling

The influence of chemicals as drivers of genetic-based variation in dependent arthropod and fungal community composition in *E. globulus* was studied in a common environment field trial, located at Salmon River (41° 010’ S, 144° 520’ E) in north-west Tasmania, Australia. In brief, individual trees in the trial were grown from open-pollinated seed lots (families) collected from 140 native

trees sampled from across the natural range of *E. globulus*. The trial was planted in 2006 as a randomized incomplete block design consisting of 25 replicates and 13 incomplete blocks per replicate, with families randomized as single-tree plots within each replicate. Full trial details can be found in Hamilton *et al.* (2013). For the present study, a total of 246 trees were sampled originating from 13 geographic sub-races, of *E. globulus*, with 7-13 families per sub-race and two individuals per family. A total of 30 fully expanded adult leaves were collected from each sampled tree during the last and first week of March and April 2012, respectively. Leaves were randomly sampled from branches felled from the north side of each tree. Twenty leaves were placed in paper bags for community assessment and 10 sealed in plastic bags for assessment of foliar chemistry. The leaves sampled for community assessment were stored at 30°C to dry prior to assessment, while the leaves for foliar chemistry were stored at -20°C until chemical extraction.

5.2.2 Community assessment

The foliar arthropod (primarily herbivorous arthropods) and fungal community was assessed using a symptom-based approach (Barbour *et al.* 2009c; DeWoody *et al.* 2013; Keith *et al.* 2010; Rönnberg-Wästljung *et al.* 2006; Tack *et al.* 2012). As *E. globulus* is grown in plantations in Australia, the symptoms of its many native insect and disease pests are well-characterized (Loch and Floyd 2001; Smith *et al.* 2005). Individual symptoms were classified through consultation with entomologists to the genus and species level where possible. In the absence of fruiting bodies and DNA testing of fungal lesions (Glen *et al.* 2007), symptoms assigned to the Tasmanian species of the genus *Teratosphaeria* (previously included in *Mycosphaerella*; Crous *et al*. 2007) are considered *Teratosphaeria*-like, and morphologically different variants treated as separate symptoms. The symptom specifically ascribed to *Teratosphaeria cryptica* was visually the same as that detailed in (Park and Keane 1982). In total, 39 individual symptoms were identified with each one scored as the presence/absence on each of the 20 leaves per tree (Table 5.1; see Appendix D Fig. 5.S1 for photographs of individual symptoms). 41% of symptoms were unique and assignable to individual taxa, and 59% were likely different symptoms of the same or different life history stage of the same taxa but maintained for analysis. The community data presented in this chapter is a subset of that from the Salmon River trial presented in chapter 2 to match the trees sampled for foliar chemistry from chapter 4.

5.2.3 Quantification of chemical profiles

The foliar wax and oil extraction and quantification was done using methods described in Gosney *et al.* (2016). In brief, waxes and oils were extracted by immersing 1g of wet leaf cut into 6mm disks from a pool of the 10 leaves in 10 ml of dichloromethane containing 100 mg heptadecane (C17) per litre as an internal standard, which was repeated two more times on the same leaf material resulting in a pooled 30ml of extract per sample. Wax and oil extracts were analyzed by gas chromatography-mass spectrometry using a Varian 3800 GC coupled to a Bruker-300 triple quadrupole MS. Waxes and terpenes (nine monoterpenes and seven sesquiterpenes) were identified based on an 'in-house' MS database of wax and oil compounds, along with the Kovats' retention index of the analysis. Individual wax compounds are expressed as heptadecane equivalents and individual terpenes as cineole equivalents on a milligram per gram of leaf dry matter basis. Leaf dry matter per sample was determined using surplus leaf material oven-dried for 7 days at 60°C. In total, 29 foliar compounds were quantified, consisting of 13 cuticular waxes and 16 foliar terpenes (Table 5.2). Of the identified cuticular waxes, nine aliphatic esters, 2 aliphatic β-diketones and 2 flavonoids were quantified and the data analyzed was the same as presented in Gosney *et al.* (2016). Of the identified foliar terpenes, nine monoterpenes and seven sesquiterpenes were quantified.

5.2.4 Data analysis

In order to determine genetic-based difference in individual foliar compounds, preliminary mixed model univariate analyses were undertaken for each trait using the package *asreml* in R. The results for cuticular waxes were originally reported in Gosney *et al.* (2016). Models for the univariate analysis followed model I presented in Table 3. To determine genetic-based differences in individual community symptoms, non-parametric Kruskal-Wallis tests based on family means were undertaken for each of the 39 symptoms using the function *kruskal.test* from the base *stats* package in R. Calculation of the variation in the dependent community composition among samples was summarized using a Bray-Curtis dissimilarity matrix obtained in Primer 6 (version 6.1.3; Roborough, Plymouth, UK). Individual symptoms were standardized by unit maxima in Primer 6, prior to construction of the Bray-Curtis dissimilarity matrix. In order to examine the influence of individual foliar waxes and terpenes on the variation in dependent community composition, additional Permanova+ analyses were undertaken fitting each compound

individually as a covariate in separate analyses. Individual wax and terpene compounds were standardized by total wax yield and total oil yield, respectively, prior to inclusion as covariates. All significant compounds from the independent Permanova+ analyses were included in relevant subsequent analyses. Following the individual compound analyses, a total of four Permanova+ analysis models were undertaken to determine the relative combined influence of the significant foliar waxes and terpenes, respectively, on the dependent community composition in relation to genetics (Table 5.3). Model I included only genetic factors and experimental design features as a baseline model for comparison with further models including the significant waxes and terpenes. The relative influence of factors and chemical covariates were quantified from the variance components obtained from the four models as the percent of explained variation in relation to the total variation for each respective model. The percent relative influence of waxes and terpenes in their respective models are reported as the sum of each compound in the model. The degree to which waxes and terpenes account for the genetic-based sub-race differences in the dependent community was calculated as the difference in the explained sub-race variation between model I and that of models II, III and IV. The change from model I to model II shows the sub-race level impact of waxes, model I to model III is that of the terpenes and model I to model VI is that of the waxes and terpenes combined. All Permanova+ analyses were done using type I sums of squares.

A canonical analysis of principal coordinates (CAP) maximizing sub-race variation was undertaken with the above Bray-Curtis dissimilarity matrix, using *capscale* from the package *vegan* in R software version 3.2.0 (R Core Team 2013). Sub-race differences were summarized through ordination of sub-race centroids from the first two CAP axes. To examine the influence of individual symptoms on sub-race differentiation, sub-race mean organism abundances were fit as vectors into the CAP ordination with *envfit* from the package *vegan* in R. Only symptoms showing a significant sub-race effect from the univariate analyses were fit as vectors in the CAP ordination. Similarly, sub-race means of standardized individual compounds found to be significant covariates in the Permanova+ analyses were fit as vectors to discern their relative influence on sub-race differences in community composition. To further investigate the association of sub-race differences in foliar wax and terpenes with the dependent community composition, independent procrustean correlations were undertaken comparing sub-race level Manhattan distance matrices of waxes and terpenes with a sub-race level Bray-Curtis community dissimilarity

matrix. All three dissimilarity matrices were obtained from sub-race mean standardized values using *vegdist* from the package *vegan* and the procrustean correlation analyses undertaken using *protest* from the package *vegan* in R.

In order to further explore the relationship of foliar waxes and oils and the dependent arthropod and fungal community, sub-race level correlations were undertaken using Bayesian inference between significant foliar compounds from the Permanova+ analyses and individual symptoms. Bayesian correlations were performed using JAGS version 4.1.0 (Plummer 2003) through the package *R2jags* in R on sub-race arithmetic means, with initial values set as the results of a Pearson correlation using *cor* from the base *stats* package in R for each combination. All Bayesian correlations were run with three chains, a thinning of 1, a burnin of 10000, and 100000 iterations. Significant correlations were designated as those for which the 95% confidence intervals did not overlap zero.

Table 5.1. Grand means, standard deviation (SD) at the individual tree level (n=246), and the χ^2 values and probability (p) for the difference among sub-races from a non-parametric Kruskal-Wallis analysis of family means (n=123) for the assessed foliar arthropod and fungal symptoms in a common environment field trial of *Eucalyptus globulus*.

Symptom[a]	Guild[b]	MEAN[c]	SD[d]	χ^2 [e]	p[e]
Paropsisterna sp. (adult)	C	0.88	0.11	13.29	0.348
Aulographina eucalypti 1	F	0.74	0.25	17.40	0.135
Teratosphaeria 1 (like *cryptica*)	F	0.50	0.21	28.53	0.005*
Plesanemma fucata	G	0.41	0.19	19.70	0.073
Teratosphaeria. 2 (following arthropod damage)	F	0.39	0.23	20.45	0.059
Gonipterus complex	C	0.22	0.13	20.55	0.057
Aulographina eucalypti 2	F	0.18	0.18	13.85	0.310
Cadmus excrementarius 1	C	0.09	0.12	24.21	0.019*
Teratosphaeria. 3 (and necrosis)	F	0.05	0.07	14.44	0.273
Cadmus excrementarius 2	C	0.05	0.08	22.66	0.031*
Pseudocercospora 1	F	0.04	0.11	9.07	0.697
Teratosphaeria. 4	F	0.02	0.04	9.76	0.637
Coleoptera 1	C	0.02	0.04	9.30	0.677
Cadmus cognatus	C	0.02	0.07	13.75	0.317
Teratosphaeria 5 (following arthropod damage)	F	0.02	0.07	11.97	0.448
Pseudocercospora 2	F	0.01	0.09	13.17	0.357
Coleoptera 2	C	0.01	0.04	6.54	0.887
Coleoptera 3	C	0.01	0.07	8.08	0.779
Ophelimus & *Myllorhinus dentifer*	S	0.01	0.06	7.33	0.835
Glycaspis cameloides (1st instar)	S	0.01	0.02	22.02	0.037*
Lepidoptera: *Psychidae*	G	0.01	0.03	14.24	0.286
Teratosphaeria 6	F	0.01	0.02	18.37	0.105
Teratosphaeria cryptica	F	0.01	0.04	11.30	0.503
Pseudocercospora. 3	F	0.00	0.03	9.84	0.630
Teratosphaeria 7 (following *Eurymeloides bicincta*)	F	0.00	0.02	9.00	0.703
Lepidoptera grazer (followed by necrosis)	G	0.00	0.02	8.18	0.771
Teratosphaeria. 8 (and necrosis)	F	0.00	0.02	19.96	0.068
Hymenoptera	G	0.00	0.01	22.59	0.031*
Paropsisterna agricola (egg cases and larval moult)	C	0.00	0.01	21.94	0.038*
Lepidoptera: Leaf miner	G	0.00	0.01	15.89	0.196
Acari 1	S	0.00	0.01	16.25	0.180
Pachysacca samuelii	F	0.00	0.01	13.57	0.329
Teratosphaeria 9 (early stage)	F	0.00	0.01	26.79	0.008*
Acrocercops laciniella	G	0.00	0.01	7.55	0.819
Coleoptera 4	C	0.00	0.01	7.91	0.792
Acari 2	S	0.00	0.01	12.92	0.375
Nepticulidae	G	0.00	0.01	9.93	0.622
Schedotrioza	S	0.00	0.01	12.63	0.396
Teratosphaeria 10 (linked subepidermally)	F	0.00	0.01	9.14	0.691

[a] The number after a symptom refers to a symptom with a different form induced by the same organism (See Fig. S1 for photographs of symptoms).

[b] Damage type guild for individual symptoms consisting of fungal pathogens (F), leaf chewers (C), leaf grazers (G) and sap suckers (S).

[c] Grand means are calculated as the average proportion of 20 leaves affected by the symptom per tree.

[d] SD is the standard deviation of the grand means for each symptom.

[e] χ^2 is the value for the sub-race effect produced by *kruskal.test* in R (R Core Team 2013).

[f] P is the significance of the χ^2 values for the sub-race effect. Asterisks indicate significance ($p<0.05$). None of these differences were significant after a Bonferroni adjustment of the p-value to P=0.0013.

Table 5.2. Grand means, standard deviation (SD), and results of sub-race effects from a mixed model univariate analysis (F and p) of the assessed cuticular wax and foliar terpene compounds at the individual tree level (n=246) in a common environment field trial of *Eucalyptus globulus*

Compound	Code	MEAN[c] (mg/gDM)	SD[d]	F[e]	P[f]
WAXES[a]					
Aliphatic esters					
Benzyl n-tetracosanoate (C24)	Benzyl C24	0.08	0.08	61.5	**0.000***
Benzyl n-hexacosanoate (C26)	Benzyl C26	0.20	0.16	91.7	**0.000***
Benzyl n-octacosanoate (C28)	Benzyl C28	0.13	0.11	55.7	**0.000***
Phenylethyl n-eicosanoate (C20)	Phenylethyl C20	0.01	0.01	18.9	0.090
Phenylethyl n-docosanoate (C22)	Phenylethyl C22	0.02	0.02	15.4	0.221
Phenylethyl n-tetracosanoate (C24)	Phenylethyl C24	0.07	0.05	18.5	0.100
Phenylethyl n-pentacosanoate (C25)	Phenylethyl C25	0.01	0.01	23.0	0.028*
Phenylethyl n-hexacosanoate (C26)	Phenylethyl C26	0.10	0.07	21.5	0.043*
Phenylethyl n-ocatcosanoate (C28)	Phenylethyl C28	0.07	0.05	29.7	**0.003***
Aliphatic B-diketones					
n-Hentriacontane-14, 16-dione (C31)	β-diket C31	0.16	0.15	74.2	**0.000***
n-Tritriacontane-16, 18-dione (C33)	β-diket C33	4.64	2.47	12.3	0.419
Flavonoids					
Desmethyl eucalyptin	des euc	0.09	0.06	41.9	**0.000***
Eucalyptin	euc	0.21	0.13	46.8	**0.000***
Total Wax Yield		5.48	2.89	16.4	0.174
TERPENES[b]					
Monoterpenes					
α-Pinene	*a*-pin	3.42	0.61	42.7	**0.000***
ρ-Cymene	*p*-cym	0.21	0.29	71.9	**0.000***
Limonene	lim	1.04	0.20	53.6	**0.000***
1,8-Cineole	cineole	15.19	3.37	82.6	**0.000***
trans-Pinocarveol	*trans*-pin	0.04	0.03	26.9	0.008*
α-Terpineol	*a*-terp	0.29	0.12	57.6	**0.000***
Nerol	nerol	0.01	0.01	28.7	0.004*
Terpinyl acetate	terp ace	0.41	0.43	45.6	**0.000***
Neryl acetate	neryl ace	0.02	0.02	24.1	0.019*
Sesquiterpenes					
Aromadendrene	aroma	3.29	0.80	44.1	**0.000***
Alloaromadendrene	alloaroma	0.63	0.13	55.2	**0.000***
Bicyclogermacrene	bicyclo	0.25	0.05	39.4	**0.000***
Globulol	glob	1.64	0.42	43.9	**0.000***
Viridiflorol	viri	0.43	0.12	43.3	**0.000***
β-eudesmol	β-eudes	1.35	2.30	205.8	**0.000***
Eudesmyl acetate	eudes	0.37	0.72	197.5	**0.000***
Total Oils		28.61	9.75	110.2	**0.000***

[a] Waxes are expressed heptadecane equivalents.

[b] Terpenes are expressed as 1,8-Cineole equivalents except for 1,8-Cineole, which is expressed as milligrams per gram DM.

[c] Grand means are calculated from the total yield of waxes and terpenes independently.

[d] SD is the standard deviation of the grand means for each compound.

[e] F is the Wald-F value for the fixed sub-race effect produced from a mixed model analysis for each compound using the package *asreml* in R. Values for the cuticular waxes was previously published in Gosney *et al.* (2016).

[f] P is the significance of the Wald-F for the fixed sub-race effect. Asterisks indicate significance ($p<0.05$), with boldface indicating significance after a Bonferroni adjustment of the p-value to P=0.0035 for cuticular waxes and P=0.0031 for foliar terpenes.

Table 5.3. Statistical models representing the order of the effects in the type I Sum of Squares Permanova+ analyses.

Model	Description
I	y = μ + *Rep* + **Sub-race** + *Family(Sub-race)* + Residual
II	y = μ + *Rep* + Waxes + **Sub-race** + *Family(Sub-race)* + Residual
III	y = μ + *Rep* + Terpenes + **Sub-race** + *Family(Sub-race)* + Residual
IV	y = μ + *Rep* + Waxes + Terpenes + **Sub-race** + *Family(Sub-race)* + Residual

Fixed effects are in boldface, random effects are in italics and covariates are in regular font.

5.3 Results

Consistent with Barbour *et al.* (2009c), we found significant variation in dependent community composition between sub-races of *E. globulus*, accounting for 3.48% of the community variation among trees in the present study (Table 5.4). The random family within sub-race variation and spatial variation (ie. *Rep*), were not significant in any of the four analyses. The lack of family within sub-race significance is likely due to low replication within families. Sub-race level procrustean analyses showed significant correlations of the community dissimilarity matrix with both the wax ($r=0.73$, $p<0.001$) and terpene ($r=0.73$, $p<0.001$) distance matrices. Of the 29 foliar compounds, four waxes and seven terpenes were found to be significantly associated with the dependent arthropod and fungal community composition (See Figure 5.1), all of which had shown sub-race differences in the univariate analyses (Table 5.2). At the individual tree-level, compared with Model 1, significant waxes explained an addition 2.4% of the variation amongst trees whereas the significant terpenes explained an additional 7.6% of the variation compared when fitted separate to the waxes (Table 5.4). When fitted after the significant waxes, the terpenes explained an additional 6.3% of the variation amongst trees, and the significance of both wax and terpenes in Model V argue they explain independent facets of the community variation amongst trees with the terpenes explaining a greater component of the variation. However, the waxes and terpenes equally explained components of the sub-race variation. Inclusion of significant waxes in Model II of the Permanova+ analyses accounted for 22.9% of the original sub-race variation (i.e. the variance explained by sub-race dropped from 3.5 to 2.7%), which was the same as the reduction observed with the significant terpenes in Model III (Table 5.4). In combination (Model IV), wax and terpenes accounted for 8.6% of total community variation amongst trees and 34.3% the sub-race variation. However, even together they clearly did not fully explain sub-race differences in

dependent community composition, with statistically significant ($p<0.01$) sub-races effects still evident in Model V.

Table 5.4. Proportion of the variation in the dependent community composition that is explained by factors through variance partitioning of the Bray-Curtis dissimilarity matrix using Permanova+ analyses.

Effect	Model I	Model II	Model IV	Model V
Replicate	0.7^{NS}	0.7^{NS}	0.7^{NS}	0.7^{NS}
Waxes	-	2.4*	-	2.3*
Terpenes	-	-	7.4*	6.3*
Sub-race	3.5***	2.7**	2.7***	2.3**
Family(Sub-race)	2.9^{NS}	2.3^{NS}	3.9^{NS}	3.2^{NS}
Residual	92.9	91.9	85.3	85.2

Wax and oil values represent the sum of the proportional influence of these compounds in the respective models. Compounds representing these values are those found significant in the Permanova+ analyses with individual compounds. Significance of effects are indicated as follows: NS for non-significant effects; *, $p<0.05$; **, $p<0.001$, ***, $p<0.0001$. Significance of waxes and oils are presented as the average of included compounds.

Sub-race variation in dependent arthropod and fungal community composition was primarily due to differentiation between mainland Australian and Tasmanian sub-races, with the geographically intermediate Bass Strait Island sub-races more-or-less intermediate between them (Fig. 5.1). Tighter grouping of Tasmanian sub-races in the ordination space indicate a more similar community between them compared to the mainland Australian sub-races. Differentiation between mainland and Tasmanian sub-races appears to be primarily due to differences in abundances of *Cadmus excrementarius* (1 and 2) and *Teratosphaeria sp*. 6 (Fig. 5.1; bottom right). Of the 11 significant foliar compounds, the community differences between the mainland Australian sub-races and those of the Tasmanian sub-races was most aligned with vectors associated with relative decreases in levels of the wax compound - C31 β-diketone, and the terpene - bicyclogermacrene, and increases in the terpenes - *trans*-pinocarval, ρ-cymene, eudesmol and β-eudesmol (Fig. 5.1; top right). Of these compounds, C31 β-diketone has previously shown evidence of being under divergent selection in *E. globulus* (Gosney *et al.* 2016). Differentiation between the Tasmanian and the Bass Strait island sub-races is mainly due to relative abundances of the well-studied leaf pathogen *Teratosphaeria sp*. 1. While not significant, the only compound associated with this differentiation is the cuticular wax, benzyl alkanoate C28, which has been previously implicated

as being bioactive in *E. globulus* (Jones *et al.* 2002). Similarly, benzyl alkanoates C24 and C26 have also shown evidence for bioactivity and while only C24 presented significance in the ordination space, both compounds appear to be associated with differences between mainland Australian and Bass Strait island sub-races. The differences between the mainland Australian and Bass Strait island sub-races in this study appear to be mainly due to relative abundance of *Teratosphaeria sp*. 9.

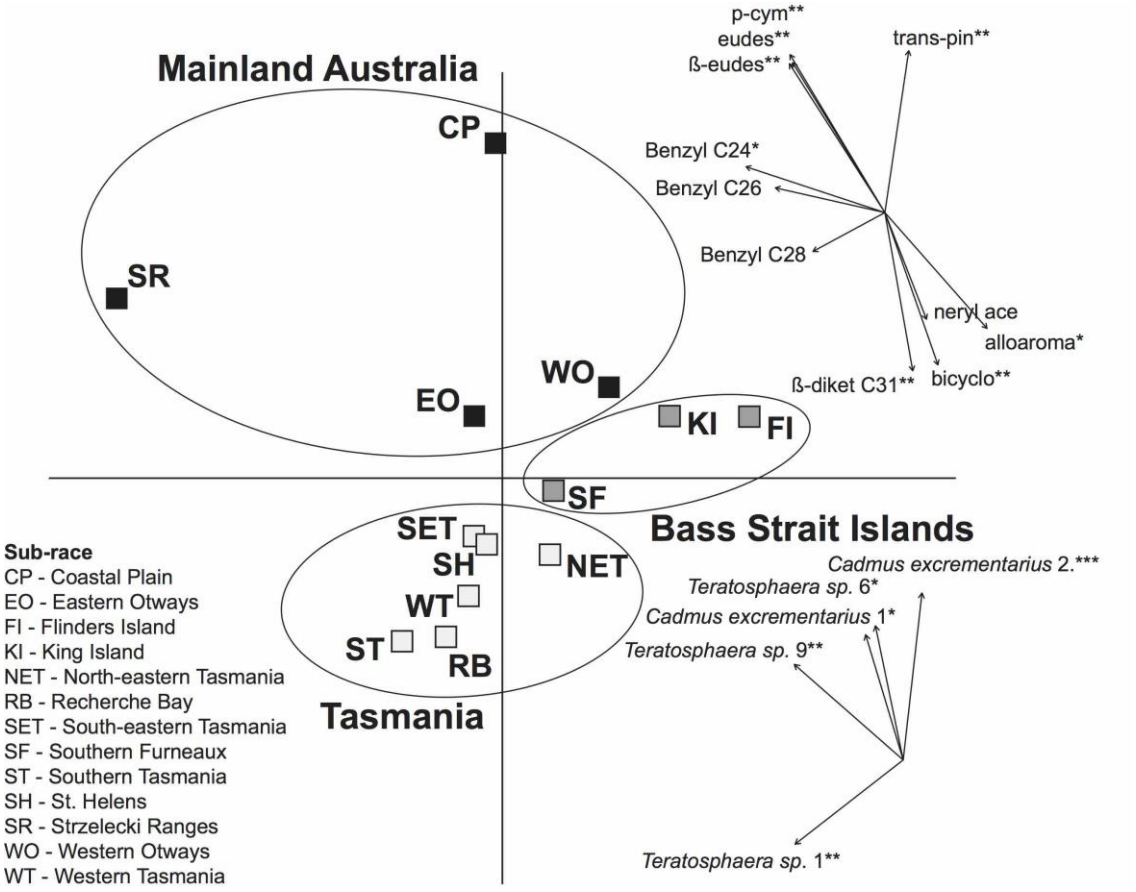

Fig. 5.1. CAP ordination summarizing the variation in dependent community composition between geographic sub-races of *Eucalyptus globulus*. Sub-races codes are presented in bottom left corner of the plot. Black squares represent mainland sub-races, grey squares are Bass Strait island sub-races and white squares are Tasmanian sub-races. The fitted vectors for individual symptoms (bottom right) as well as compounds found significant in the individual compound Permanova+ analyses (top right) are presented. Significance of vectors are indicated as follows: NS for non-significant effects; *, $p<0.05$; **, $p<0.001$, ***, $p<0.0001$.

Of the 39 assessed symptoms, seven were significantly correlated with wax compounds at the sub-race level, while fifteen were significantly correlated with terpenes. At the sub-race level, these seven cuticular wax compounds had nine significant correlations with individual symptoms, with five negative correlations and four positive correlations (Fig. 5.2). Forty-one sub-race level correlations between individual symptoms and foliar terpenes were significant, with eleven negative correlations and thirty positive correlations (Fig. 5.2). While there are a far greater number of significant correlations of terpenes than waxes with individual organisms, most of these correlations were with rare symptoms (occurring on less than 10% of all trees) driven by the outlying Coastal Plain sub-race, which exhibited both a greater abundance of individual symptoms and higher concentrations of terpenes relative to the other sub-races. Most of these terpene correlations were no longer significant after removal of the Coastal Plain sub-race.

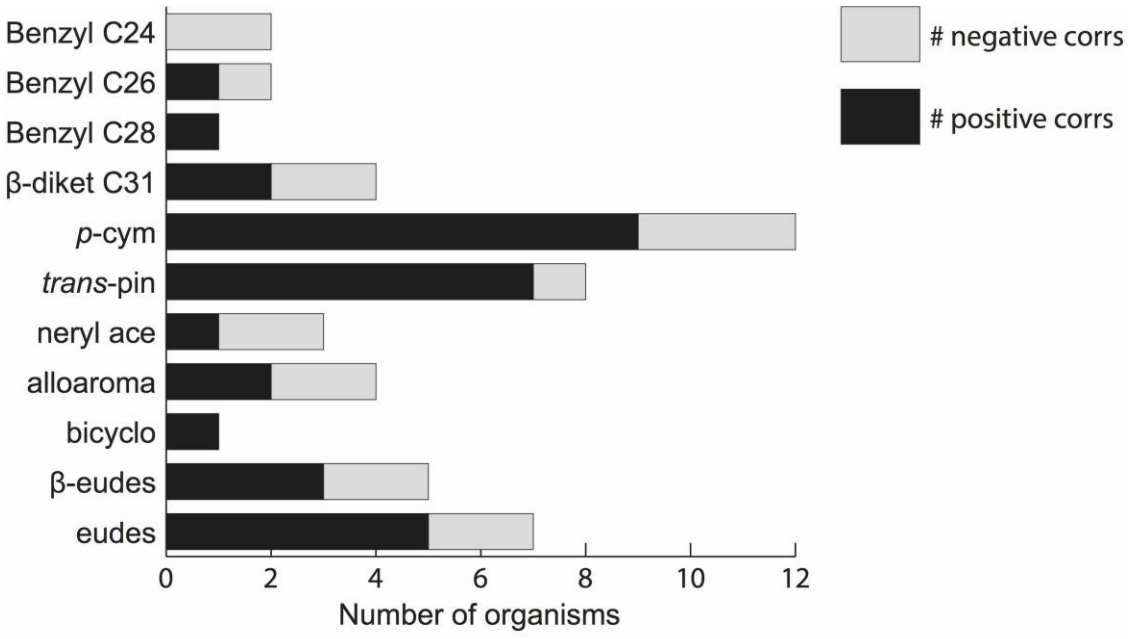

Fig. 5.2. Bar chart of significant individual symptom sub-race level correlations with cuticular wax and foliar terpene compounds found to significantly impact community composition. White bars represent negative correlations; black bars represent positive correlations. Significance was determined from the 95% confidence intervals (CI) of the Bayesian correlation results. CI's which did not overlap zero were considered significant.

5.4 Discussion

Chapter 2 showed that genetic variation in the forest tree *E. globulus* does have an extended effect on the dependent foliar arthropod and fungal community with a stable pattern of the variation across multiple trials and studies. However, the potential mechanisms driving such genetic-based patterns of variation in the dependent community were not elucidated. In the present study, we provide evidence for the phenotypic and genetic-based association of cuticular waxes with the community level variation of the dependent foliar arthropod and fungal community on the forest tree *E. globulus*. We show that cuticular waxes can explain a statistically significant component of the variation in canopy communities of *E. globulus*, and in terms of sub-race differences their effects are comparable to that of the terpenes, a well-studied group of defensive compounds of eucalypts. While cuticular waxes have long been implicated in influencing insect herbivory (Edwards 1982; Jones *et al.* 2002), to our knowledge, this is the first to suggest a community level impact. Nevertheless, potential mechanisms for such impacts are provided by previous studies for specific fungal and insect organisms. For example, waxes appear to influence oviposition choice in eucalypts (Ennis *et al.* 2016; Rapley *et al.* 2004b; Steinbauer *et al.* 2004) and have been shown to affect insect adhesion in eucalypts (Brennan and Weinbaum 2001) and other systems (Eigenbrode and Espelie 1995; Eigenbrode and Jetter 2002).

The effects of foliar terpenes on the dependent community has garnered more focus (Gershenzon and Dudareva 2007), with their influence even thought to extend to multiple trophic levels. For example, in *Arabidopsis* the parasitic wasp, *Cotesia rubecula*, is attracted to plants that display an herbivore induced production of terpenes, which in turn influence the abundance of the wasps specialized prey, *Pieris rapae* (Van Poecke *et al.* 2001). Many of the correlations of foliar terpenes with individual organisms in this study were positive associations, which could reflect attraction cues for particular organisms (Katerinopoulos *et al.* 2005), but is difficult to unravel given the often inter-correlated biochemical pathways involved (O'Reilly-Wapstra *et al.* 2011). While attraction cues for insect predators have not been examined in eucalypts, foliar terpenes have been implicated as defensive compounds to mammalian browsing (O'Reilly-Wapstra *et al.* 2004; Padovan *et al.* 2013). This may provide a basis for the indirect effects of foliar terpenes on the dependent community in eucalypts as well, given mammalian browsing has previously been

implicated in influencing the dependent arthropod and fungal community in *E. morrisbyi* (Gosney *et al.* 2014).

Variation in canopy communities tended to be associated more with the phenotypic variation in minor rather than major components of the cuticular wax and terpene profiles. The two respective primary components of the wax and terpene profiles, C33 β-diketone and 1,8-cineole respectively, showed no significant association with variation in the dependent community. While lack of significance of C33 β-diketone may not be very surprising as it previously showed no significant variation at the sub-race level in *E. globulus* (Gosney *et al.* 2016), the absence of an association of 1,8-cineole with variation in the dependent foliar community is unexpected, particularly given the highly significant sub-race differences. In eucalypts, 1,8-cineole has been the primary terpene implicated in the defense of both insect and mammalian herbivory, which has been consistent across multiple species (Edwards *et al.* 1993; Matsuki *et al.* 2011; O'Reilly-Wapstra *et al.* 2004). Increasing concentration of cineole in six species of eucalypts has been significantly associated with increased resistance to Christmas beetles (Edwards *et al.* 1993), which were not present in the community assessed in this study. Of the minor compounds significantly associated with the dependent community in this study, only the benzyl alkanoates in the wax profile have shown previous evidence of bioactivity in eucalypts. In this case, *Mnesampela private* (Autumn gum moth) susceptible phenotypes of juvenile *E. globulus* displaying lower concentrations of these compounds (Rapley *et al.* 2004b). While *Mnesampela private* was not present in this study as the insect primarily affects juvenile foliage rather than the adult foliage assessed here, our study suggests that the bioactivity of some of these compounds may extend beyond an individual organism, influencing the broader composition of the dependent community. Additionally, all cuticular waxes showing community level effects in this study have shown signals of divergent selection in *E. globulus* (Gosney *et al.* 2016). This suggests that adaptive variation could have extended effects at the community level.

In the present study, genetics-based sub-race differences, cuticular waxes and foliar terpenes combined accounted for nearly fifteen percent of the variation in the dependent community composition of *E. globulus*, however, more than eighty-five percent of the overall variation is still unexplained. Independently, both the waxes and terpenes have been shown to be highly inter-

correlated within their respective classes (Gosney *et al.* 2016; O'Reilly-Wapstra *et al.* 2011). There may be interactions between these compounds impacting the community in ways we were unable to detect in the current study. Such interactions may be the result of the biosynthesis of these compounds in which some of these compounds may be influencing the community earlier on or during the juvenile and transitional stages of *E. globulus* (Borzak *et al.* 2014). In addition to the compounds in the current study, other foliar chemistry not assayed may explain a proportion of the unaccounted variation. Likely candidates are other secondary metabolites, such as formylated phloroglucinol compounds (FPCs), which have been shown to influence insect herbivory in eucalypts (Henery *et al.* 2008; Matsuki *et al.* 2011), and have shown a community influence in *E. globulus* (Barbour *et al.* 2009c), and condensed tannins, which have a known bioactive history in numerous plant systems (Bailey *et al.* 2006; Barbour *et al.* 2009c; Brunet *et al.* 2008; Schweitzer *et al.* 2008b; Whitham *et al.* 2006). In eucalypts, condensed tannins have been implicated in providing resistance to insect herbivory (Macauley and Fox 1980), as well as being directly related to the dependent foliar arthropod and fungal community in *E. globulus* (Barbour *et al.* 2009c). Besides foliar chemistry, a portion of the unexplained variation may be due to additional tree characteristics, such as morphological traits (e.g. SLA, leaf thickness, etc.) and overall tree architecture (Barbour *et al.* 2015; Barbour *et al.* 2009c; Steinbauer *et al.* 1998).

In conclusion, this study suggests that genetic-based variation in cuticular wax compounds can have an extended effect on dependent communities. Further, it suggests that the effects of these compounds are comparable to that of the well-studied terpenes in explaining genetic-based sub-race differences in the composition of dependent communities. Despite statistically significant genetic-based differences in many compounds, only 44.4% of the waxes and 43.7% of the terpenes also showed significant genetic-based associations with community compositional variation. All four cuticular wax compounds showing such effects had previously been reported as showing signals of diversifying selection and/or as having bioactivity (Gosney *et al.* 2016; Jones *et al.* 2002), arguing that adaptation may have extended effects. While this study suggests an important role for cuticular waxes in explaining community genetic variation, the predictability and mechanisms by which these compounds impact dependent communities are still unknown. Additionally, both cuticular waxes and terpenes only accounted for a small portion of the variation

in the dependent community, highlighting the complex nature of the drivers of extended genetic effects in forest trees.

5.5 Acknowledgements

We thank Paul Tilyard, and Justin Bloomfield for collection of the samples involved in this study. Tim Wardlaw and Caroline Mohammad for help in the identification of symptoms. Peter Harrison for symptom assessment. Noel Davies and Hugh Fitzgerald for the analysis and quantification of the chemistry involved in the study. Forestry Tasmania for access to the trials. This study is part of a PhD undertaken by Benjamin Gosney, supported by an International Postgraduate Scholarship provided by the University of Tasmania and funded by the Australian Government. Data collection was funded by Australian Research Council Discovery grants (DP140102552 and DP0773686) and a Linkage grant (LP120200380 in partnership with Greening Australia).

Chapter 6: Bioactive QTL and evidence for emergent genetic effects on the dependent arthropod and fungal community of a forest tree

Abstract

Numerous studies have shown that within species genetic variation in forest trees can affect dependent organisms and biotic communities; however, little is known of the genomic regions with community-level effects. We identify regions of the genome associated with dependent community-level effects in the forest tree *Eucalyptus globulus* and compare them to the regions associated with the individual community members using quantitative trait loci (QTL) analysis. The dependent canopy community was quantified based on forty-eight arthropod and fungal symptoms on adult foliage of samples collected from 112 clonally replicated genotypes (224 trees) of a full-sib outbred F_2 population of *E. globulus* in a common garden trial. Fifteen QTL were found for community-level effects and a total of fifty-three for individual community members. All community-level QTL detected in this study were independent of QTL affecting individual community member and are thus emergent genetic effects. Additionally, except for QTL for two foliar terpene compounds which have no evidence of arthropod and fungal bioactivity in *E. globulus*, community-level QTL are independent from previously reported QTL for foliar chemistry from the same F_2 population. While providing evidence of emergent genetic effects on canopy communities in this *E. globulus* F_2 population, this study highlights the complexity underlying phenotypic variation in community traits in forest trees.

6.1 Introduction

Emergent properties are properties of systems that cannot be fully explained by their individual components (Mayr 1982). In terms of biological research, this can be equated to properties of biological systems that are not easily measurable or understood based on the predictability of their components (Müller 1996). For example, increasing levels of biological hierarchy, such as cells, organs, organisms, communities and ecosystems are thought to be emergent from one level to the next (Nielsen and Müller 2000). This emergence is based on complex interaction networks at lower

levels, which arise to form the next level in the hierarchy (Reuter *et al.* 2005). In ecology, stability is a well-recognized emergent property of communities arising from complex interactions between community members and their environment (Brown *et al.* 2001; Keith *et al.* 2010). In a broader sense, other community parameters, such as diversity and community composition, may also themselves be emergent. For example, despite drastic changes in desert rodent community composition due to extinction and colonization events over a 22-year period, the average rodent species richness and abundance was relatively stable (Brown *et al.* 2001; Ernest and Brown 2001). In this regard, community parameters can be viewed as emergent properties of processes which cannot be entirely understood based on knowledge of the individual community members (Brown *et al.* 2001). Additionally, there is evidence that such emergent properties may be genetically based. In the forest tree *Populus angustifolia* for example, the seasonal stability of the variation in arthropod community composition among clonally replicate genotypes was shown to be moderately heritable (H^2=0.32; Keith *et al.* 2010).

A genetic basis to variation in dependent communities has been well established in numerous plant systems, suggesting there are genes impacting on community-level responses (Whitham *et al.* 2012). In foundation species, such as trees, plant genetic variation has been shown to influence multiple levels of the dependent community, from individual organism abundances to organism richness and overall composition (Gosney *et al.* 2014; Maddox and Root 1987; Whitham *et al.* 2006; Wimp *et al.* 2007). These extended genetic effects can have diverse impacts, from the below-ground microbial communities (Schweitzer *et al.* 2008a) to the bark invertebrate (Barbour *et al.* 2009b) and foliar herbivore communities (Whitham *et al.* 2006). Additionally, the influence of genetic variation in forest trees on the dependent community has been reported at different genetic scales, including among provenances within a species (Barbour *et al.* 2009c; Gosney *et al.* 2014; Sinclair *et al.* 2015), families within provenances (Chapter 2; Axelsson *et al.* 2015), as well as QTL (DeWoody *et al.* 2013; Rönnberg-Wästljung *et al.* 2006) and specific genes (Zinkgraf *et al.* 2016). From this community genetics perspective, the impact of emergent properties of communities has potential evolutionary implications as community-level effects may be under independent natural selection from their biotic components.

In community genetics, individual organisms appear to explain, in part, properties of community-level variation. For example, underlying provenance-level trends in genetic based variation in arthropod and fungal symptom richness and community composition in a forest tree, *Eucalyptus globulus*, are the same as those associated with a few dominant organisms in the community (Chapter 2). These trends are also apparent in numerous foliar chemical compounds (Gosney *et al.* 2016; O'Reilly-Wapstra *et al.* 2013b), with some reported to have significant associations with individual organism and dependent community responses (Chapter 5; Jones *et al.* 2002). At the genomic-level, most of the quantitative trait loci (QTL) for arthropod functional guild community abundances in the forest tree hybrid *Populus trichocarpa* × *P. deltoides* co-located with genes involved in the biosynthetic pathways of known defensive compounds (DeWoody *et al.* 2013). To date, the study by DeWoody *et al.* (2013) and that of Rönnberg-Wästljung *et al.* (2006) investigating QTL for resistance to general damage types associated with various insect and game species in the willow *Salic dasyclados* are the only studies to examine QTL influencing dependent community-level responses. While these studies suggest that genetic-based variation in community parameters may not be emergent, no study has identified QTL for community parameters and individual members of the community, which would allow for direct comparison.

Here we examine the regions of the genome influencing the dependent arthropod and fungal community of *Eucalyptus globulus* using a QTL approach. The forest tree *Eucalyptus globulus* is part of the family *Myrtaceae* and is native to southeastern Australia. It is widely grown in temperate regions of the world for commercial purposes and genetic variation within the species has been studied extensively (Gosney *et al.* 2016; Jones *et al.* 2013; Potts *et al.* 2004). Extended genetic effects in the species have been shown in numerous studies and at multiple genetic scales with a degree of genetic based stability in community traits evident across environments (Chapter 2). While QTL have been identified that influence tree susceptibility to a major arthropod pest (*Mnesampela privata* – Jones *et al.* 2002) and fungal pathogens (*Puccinia psidii* - Butler *et al.* 2016; *Mycosphaerella cryptica* - Freeman *et al.* 2008b) known to cause substantial damage in *E. globulus*, no study to date has examined QTL associated with community-level traits. In this study, we take a genetic approach to testing the hypothesis that communities do have emergent properties, arguing this will be signaled by the relative independence of at least some of the QTL for community parameters from those of the individual community members. Additionally, we

compare the location of QTL for community parameters and individual community members with previously reported QTL for foliar chemistry, including compounds with reported bioactive roles in *E. globulus*.

6.2 Methods and materials

6.2.1 Field trial & sampling

The arthropod and fungal community assessment and subsequent QTL analyses were undertaken on the adult foliage of a full-sib outbred F_2 population consisting of 112 clonally replicated genotypes (224 trees). This mapping population was generated by crossing F_1 parents derived from unrelated trees from King Island (located in the Bass Strait north of Tasmania) and Taranna, located in southeastern Tasmania based on their resistance/susceptibility to *Mycosphaerella* leaf disease (see Freeman *et al.* 2006). The trees in this study were planted in a common environment field trial located at Woolnorth (40°52'S, 144°50'E) in northwest Tasmania, Australia in May 1998. The trial comprised two randomized blocks with each genotype represented once per block. Full trial details can be found in Milgate *et al.* (2005b). Adult foliage was sampled from all 224 trees in the trial between 11-29th July, 2008. Two branches were felled from the lower part of the crown on the north side of each tree. An average of nine branchlets with fully expanded adult foliage were removed from each felled branch (18 per tree), placed in large paper bags, dried and stored at room temperature until assessment.

6.2.2 Community assessment and calculation of community-level traits

A symptom-based approach was used to assess the foliar arthropod (primarily herbivorous arthropods) and fungal community on the 224 trees in this study. This approach is a widely used alternative to live organism assessment in invertebrate community studies (Barbour *et al.* 2009c; DeWoody *et al.* 2013; Gosney *et al.* 2014; Tack and Roslin 2011). Symptoms were identified to species level, where possible, based on publications and field and laboratory observations. In total, 50 symptoms (individual community members) were identified across the 224 trees with each symptom scored as the relative percent cover affected across all sampled foliage for each tree (See Appendix E Table 6.S1 for symptom descriptions). Individual scores of all assessed symptoms for each tree were used to calculate four primary community parameters and quantify the

community compositional variation between genotypes. Community parameters consisted of total abundance, symptom richness, Shannon-Weiner diversity and Pielous' evenness; calculated using the function *diversity* from the package *vegan* in R 3.3 (R Core Team 2013). Community compositional variation among trees was summarized through non-metric multidimensional scaling (NMDS), which is a well-recognized technique for reducing compositional change to independent traits in community genetics studies (Barbour *et al.* 2009c; Keith *et al.* 2010; Whitham *et al.* 2006). Prior to NMDS, community data was standardized by unit maxima for each symptom using function *decostand* from the package *vegan* in R. NMDS scores were obtained from the standardized multivariate data using the function *metaMDS* from the package *vegan* in R, using a Bray-Curtis dissimilarity index method and 50 random starts. After the calculation of community parameters and quantification of community compositional variation, genotype means (ie. mean of the two clonal trees per F_2 genotype) were calculated for all community traits and used as the phenotypic data for QTL analysis to remove some of the environmental effects and enhance the power of QTL detection.

6.2.3 QTL analysis of community traits

QTL detection was undertaken using genotype mean data and a previously reported linkage map of the F_2 pedigree (Gosney *et al.* 2016). The first linkage map in the pedigree (Freeman *et al.* 2006) was constructed mostly from amplified fragment length polymorphism (AFLP) markers. Later, diversity array technology (DArT) markers were added to the map to increase marker density and genome coverage (Hudson *et al.* 2012a). To reduce computational demand for QTL analysis a subset of 253 markers were retained from the latter (695 marker) map, chosen to maximize map coverage and spacing of markers segregating from each parent (see Gosney *et al.* 2016 for more details). QTL analysis was done using the regression approximation to maximum likelihood mapping in MAPQTL 6.0 (Van Ooijen 2009). Significance of QTL was determined at a genome-wide type I error ($p<0.05$) and chromosome-wide type I error ($p<0.05$). The procedure for interval mapping, multiple QTL model (MQM) and setting LOD thresholds for QTL significance were undertaken as previously described in Freeman *et al.* (2008a), except in this case only forward selection of cofactors was used and MQM mapping used the restricted MQM procedure (see Van Ooijen 2009). Additionally, clonal repeatability for each community-level trait and individual

symptom was calculated from linear mixed models fitted by restricted maximum likelihood (REML) with genotype fit as a random term using the function *rpt* from package *rptR* in R.

6.2.4 QTL hotspot detection

To test independence among QTL, two types of analyses were undertaken: 1) direction of segregation; and 2) hotspot detection. QTL that are independent are due to different loci and genes; QTL that are dependent are either tightly linked loci or an effect of the same locus (i.e. a gene with pleotropic effects). The logic of the direction of segregation test is that if two QTL near each other have a different direction of segregation (e.g one segregates only in the male parent while the other segregates only in the female) they are likely to be independent QTL. To estimate the segregation of QTL effects, a Kruskal-Wallis test was conducted for each trait in MAPQTL to determine if markers adjacent to each QTL that segregated solely from the male or female parent had significant effects.

Hotspot detection followed the procedures of Rae *et al.* (2009) and tested whether the frequency of co-locations of QTL peaks (within and between different trait classes) significantly departed from expectations under a random distribution. Hotspot detection was undertaken within and between different trait classes (community-level QTL; QTL for individual symptoms; and previously reported QTL for foliar chemistry in this pedigree) using a 5cM sliding window approach testing if the frequency of co-locations of QTL peaks (within and between different trait classes) significantly departed from expectations under a random distribution following Rae *et al.* (2009). Previously reported QTL for foliar chemistry in this pedigree included those for the concentration of formylated phloroglucinols (FPC; Freeman *et al.* 2008a), terpenes (O'Reilly-Wapstra *et al.* 2011) and cuticular waxes (Gosney *et al.* 2016) in juvenile foliage. While the arthropod and fungal community was assessed on the adult foliage in this study, there is good evidence that composition in foliar chemistry is relatively stable between foliage types (Gosney *et al.* 2016; Li *et al.* 1996; Li *et al.* 1997). The previously published QTL for FPCs and terpenes were based on the first linkage map constructed in the pedigree (Freeman *et al.* 2006), so these analyses were redone using the latest version of the linkage map. For FPCs, two of the three originally reported QTL were maintained as significant after the new analysis, while seven new QTL were

detected (Table 6.S2). For the previously reported terpenes, fourteen of the twenty-four QTL were maintained after the new analysis, while nineteen new QTL were detected. Additionally, many of the maintained QTL for both FPCs and terpenes shifted slightly compared to their originally reported peak positions (cM) and 95% confidence intervals along the chromosome (see Appendix E Table 6.S2). This was likely due to the greater map coverage of the new maps which meant in many cases the same genomic location would have a new cM value in the new maps. Significant co-locations of QTL peaks were determined based on 10000 permutation of peak QTL locations across all loci. QTL hotspots are designated as regions of the genome with a greater number of QTL than would be expected at random. A stepwise approach was taken to QTL hotspot analyses, beginning with within-trait class analyses (five trait-classes were tested corresponding to: community-level traits, individual symptoms, FPCs, terpenes and waxes) prior to between-trait class analyses. QTL hotspots detected during within trait class analyses were collapsed to a single QTL for subsequent between-trait class analyses. Between-trait class hotpots were collapsed for subsequent analyses which then included all trait-classes in the analysis. QTL plotting and hotspot analyses were undertaking using the package *qtlplots* in R. The package was provided by Nathan Street, an author of the paper originally describing the approach (Rae *et al.* 2009).

6.3 Results

6.3.1 Symptom variability

Fifty individual symptoms were identified in total; 15 leaf chewers, 15 leaf grazers, 11 sap suckers, 7 fungi and 2 predators. Eight percent of symptoms were rare, occurring on less than 5% of the genotypes. The fungal pathogen *Aulographina eucalypti*, the moth larvae *Doratifera oxyleyi* and the beetle Chewer 2 were the most frequent symptoms, occurring on all genotypes, followed by the snout beetle *Gonipterus scutellatus* (99% of genotypes) and beetles Chewer 3 and 4 (95% of genotypes). In terms of abundance, only three symptoms could be considered dominant, with an average percent cover per genotype greater than 50% (Table 6.1). The most abundant of these was Chewer 2 (avg. 80% cover), followed by *Doratifer oxleyi* (avg. 54% cover) and *Aulographina eucalypti* (avg. 50%). Most symptoms were far less abundant with 38 of the 50 identified symptoms showing less then 5% cover damage per genotype. Despite generally low clonal repeatability (Table 6.1), QTL were detected for most community-level traits and over half of the individual symptoms.

Table 6.1. Putative quantitative trait loci (QTL) for community-level traits and individual symptoms.

Trait	Guild[a]	LG[b]	Nearest marker[c]	Map pos (cM)	Peak LOD[d]	% exp[e]	Seg.[f]	Mean[g]	SD	Clonal repeat.
Community-level traits										
NMDS1		2	Emb158	53.4	3.0	8.0	F	-	-	0.12 ± 0.12
		5	570670	59.4	4.8*	10.7	F			
		8	568780	92.0	3.2	8.4	F			
		11	571397	61.9	7.7***	21.8	M			
NMDS2		9	640225	11.2	3.5	14.5	M	-	-	0.25 ± 0.14
NMDS3		2	CRC8	104.4	3.6	8.6	M	-	-	0.22 ± 0.13
		5	Emb208	87.8	6.2***	18.3	F			
		7	566500	23.3	4.3*	12.4	F			
		10	644030	80.9	3.5	9.9	M			
Abundance		2	Emb158	53.4	4.9*	15.6	F	233.41	47.89	0.03 ± 0.09
		6	CRC11	59.4	4.0	4.0	B			
		9	564698	8.2	4.7*	14.5	M			
Richness		5	568743	81.4	5.4**	20.1	F	16.10	2.56	0.09 ± 0.11
		11	562840	52.2	3.7	13.0	M			
Evenness		1	Emb12	70.0	3.4	15.1	F	0.62	0.05	0.13 ± 0.13
Individual symptoms										
Aulographina eucalypti	F	3	563549	111.6	3.4	15.0	M	50.23	27.19	0.27 ± 0.13
		7	p14b03	14.4	3.3	14.8	M			
Seridium eucalypti	F	9	575650	0.0	3.6	11.2	M			
		11	571397	63.5	6.3***	20.9	F			
Fungi 1	F	8	503944	135.6	3.5	15.4	F	1.57	2.29	0.15 ± 0.12
Fungi 2	F	4	641176	54.1	3.8	16.8	M	0.12	0.31	0.31 ± 0.14
Fungi 3	F	2	575243	0.0	3.5	13.4	F	1.42	1.47	0.13 ± 0.12
		3	567811	109.5	3.7	14.1	M			
Cadmus spp.	C	5	p06b11	52.8	5.2	20.3	M	3.84	4.58	0.32 ± 0.13
		10	*COBL4*	56.7	3.2	11.9	F			
Doratifera oxleyi	C	6	CRC11	59.4	4.2	18.1	M	54.07	20.73	0.07 ± 0.11
Gonipterus scutellatus (sqrt)	C	1	644046	95.8	3.4	12.6	B	7.03	6.00	0.00 ± 0.09
		11	571397	59.9	4.4	16.2	M			

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Trait	Guild[a]	LG[b]	Nearest marker[c]	Map pos (cM)	Peak LOD[d]	% exp[e]	Seg.[f]	Mean[g]	SD	Clonal repeat.
Heliotrioza	C	4	p13b15	35.3	4.0	17.6	F	0.08	0.32	0.00 ± 0.08
Hetereonyx	C	11	p15b02	28.5	4.2	18.3	M	0.08	0.79	-
Schedotrioza	C	2	568300	61.2	4.4	15.9	F	0.08	0.32	0.00 ± 0.09
		9	p15b01	12.2	6.1	20.7	M			
Chewer 1	C	5	p17b05	19.1	3.2	14.2	M	0.32	0.72	0.07 ± 0.11
Chewer 2	C	8	503819	120.8	3.2	12.4	B	80.97	19.30	0.00 ± 0.08
		9	571807	86.1	5.7*	22.6	M			
Chewer 3	C	7	566500	25.2	5.3**	20.3	F	14.09	11.50	0.36 ± 0.12
		10	566988	65.2	3.1	11.3	F			
Chewer 4	C	1	Emb12	69.0	5.6*	19.0	B	6.07	5.33	0.07 ± 0.11
		3	644451	10.7	3.5	11.4	B			
		11	p15b02	26.5	8.3*	27.1	M			
Mnesampela privata	G	9	642464	45.1	5.1	21.6	F	0.04	0.17	0.00 ± 0.09
Oecophoridae 1 (sqrt)	G	1	640754	24.9	3.8	15.5	M	2.40	2.57	0.32 ± 0.13
		8	566660	15.2	4.3*	16.0	M			
Oecophoridae 2	G	8	p10b03	68.2	3.3	14.4	B	0.08	0.28	0.00 ± 0.09
Grazer 1	G	3	*CSA3*	4.	3.1	11.4	M	0.14	0.93	0.00 ± 0.08
		11	503068	10.9	4.5	17.3	M			
Aterpus rubus	S	2	p03b02	115.1	3.8	16.7	M	0.75	0.91	0.01 ± 0.09
Ophelimus spp. (sqrt)	S	4	Emb156	21.0	4.0	9.1	F	0.42	1.09	0.10 ± 0.11
		5	575337	2.1	7.2**	17.9	M			
		6	567551	28.1	6.6**	16.2	F			
		8	566660	15.2	9.2***	24.3	M			
Phylacteophaga froggatti	S	1	Emb180	54.2	5.0*	16.5	M	3.27	3.44	0.00 ± 0.09
		3	644451	10.7	3.1	10.1	F			
		4	Emb78	14.4	4.6*	15.6	B			
Sap Sucker 1	S	4	Emb156	27.3	3.5	15.3	F	0.08	0.23	0.00 ± 0.08
Sap Sucker 2	S	11	565569	51.2	3.1	13.8	M	0.04	0.22	0.00 ± 0.09
Sap Sucker 3	S	2	573524	12.0	9.1*	35.3	F	0.04	0.17	0.00 ± 0.09
Sap Sucker 4	S	2	573524	12.0	16.5*	47.2	F	0.04	0.17	0.92 ± 0.02
		8	565854	86.3	3.2	6.5	F			
Predator 1	P	2	575243	0.0	7.6	30.5	F	0.01	0.08	0.42 ± 0.12

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[a] Damage type guild for individual symptoms consisting of fungal pathogens (F), leaf chewers (C), leaf grazers (G), sap suckers (S) and predators (P).
[b] Linkage group.
[c] Marker names beginning with Emb or CRC are microsatellites, those named p#b# are AFLP, *COBL4* and *CSA3* are candidate genes used in a previous QTL study for wood properties (Freeman *et al*. 2013) and the remainder are DArT.
[d] Peak LOD score for each QTL. Genome-wide significance is indicated by: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. The remainder were significant at the suggestive level (chromosome-wide type I error rate <0.05).
[e] The proportion of phenotypic variation between genotype means explained by each QTL.
[f] Whether the QTL segregated only from the male parent (M), the female parent (F), or both (B).
[g] Arithmetic tree means for community-level effects and individual symptoms.
[h] SD is the standard deviation of the mean for each community trait.
[i] Clonal repeatability was calculated fitting genotype as a random term in a linear mixed effect model for each community-trait.
Note: Any normality transformations are indicated in parentheses next to the name of the community trait. See Appendix E Table 6.S1 for symptom identification descriptions.

6.3.2 QTL detection

Fifteen QTL for community-level traits were identified at the chromosome-wide significance level including QTL for all community-level traits analyzed except for Shannon-Weiner diversity (Table 6.1). Each community-level QTL accounted for an estimated 4.0-21.8% of the variation between genotype means, with QTL spread throughout the genome (9 of the 11 chromosomes; Fig. 6.1). Nine QTL were detected for community composition (NMDS) across 7 of the 11 chromosomes with QTL detected for the three independent directions of change (NMDS1, NMDS2 and NMDS3). Three QTL were detected for total abundance, two for symptom richness and one for Pielous' evenness.

Forty-five QTL were identified at the chromosome-wide significance level for individual symptoms, with QTL detected for 27 of the 50 identified symptoms (Table 6.1). Each individual symptom QTL accounted for 6.5 - 47.2% of the variation between genotype means, with QTL spread throughout the genome (11 of 11 chromosomes; Fig. 6.1). QTL were detected for all three of the dominant symptoms. Two QTL were identified for *Aulographina eucalypti*, each accounting for 15% of the variation between genotype means. Two QTL were detected for Chewer 2 with the most significant accounting for 22.6% of the variation in genotype means. One QTL was detected for *Doratifera oxleyi* which explained 18.1% of the variation between genotype means.

6.3.3 QTL hotspots

Two independent hotspots were detected among community-level traits (Fig. 6.1, Table 6.2), including QTL for the two primary directions of community compositional change (NMDS1 on LG2 and NMDS2 on LG9) with total abundance. All QTL within their respective hotspots segregated from the same parent (Table 6.1). Only one between-trait class QTL hotspot including the collapsed community-level QTL hotspot on LG2 and two foliar terpenes was detected (Fig. 6.1, Table 6.2), however, both community-level QTL in the hotspot segregated solely from the female parent (Table 6.1), while both QTL for terpenes segregated solely from the male (Table 6.3). Notably, none of the community-level QTL were found in hotspots containing QTL for individual symptoms indicating that these QTL were independent from one another.

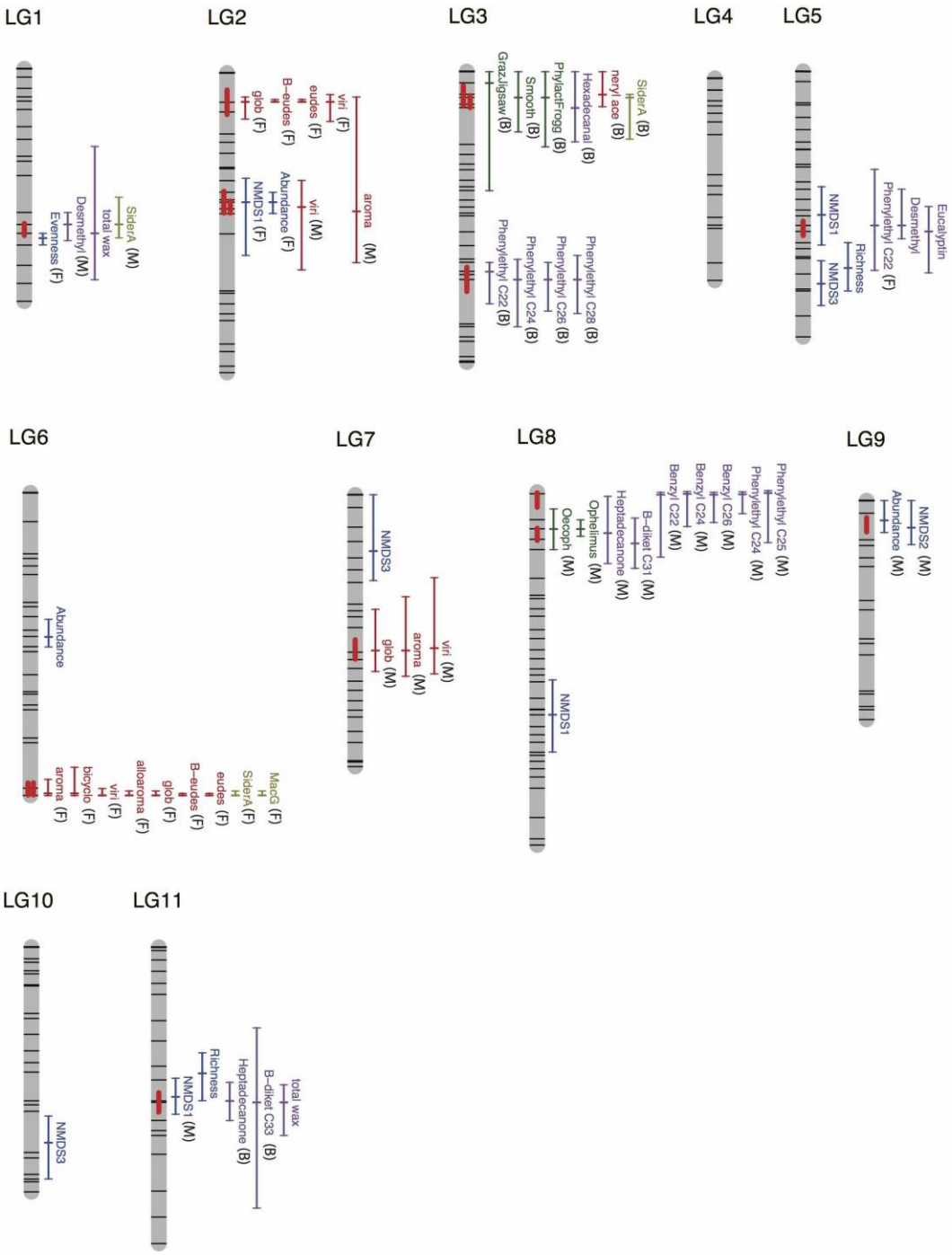


Fig. 6.1. Putative quantitative trait loci (QTL) locations for the community-level traits (blue) of adult foliage in *Eucalyptus globulus*, along with individual symptoms (green) and updated QTL for locations of FPCs (yellow), terpenes (red) and previously reported waxes (purple) of juvenile foliage present in significant QTL hotspots. QTL locations were determined through interval and MQM mapping, with peaks indicated for each QTL and two-LOD support intervals on either side of the peak, corresponding to 95% confidence intervals. Linkage group numbering and orientation follows the *Eucalyptus grandis* reference genome (Myburg *et al.* 2014). Full names for abbreviated foliar chemistry compounds are in Table 6.2. Red bars within linkage groups are statistically significant hotpots; see Table 6.3 for the list of traits in each hotspot. Black lines within linkage groups represent markers.

Table 6.2. QTL hotspots for within- and between-trait-class combinations of community-level traits, individual community members, FPCs, terpenes and waxes in *E. globulus*.

Hotspot	LG[a]	Start (cM)[b]	Stop (cM)[b]	QTL in hotspot[c]
Within-trait-class				
Community-level (C)	2	49	58	NMDS1, Abundance
	9	7	13	NMDS2, Abundance
Community members (M)	-	-	-	-
FPCs (F)	6	120	125	SiderA, MacG
Terpenes (T)	2	7	17	viri, glob, β-eud, eud
	6	120	125	allo, bicy, viri, arom, glob, β-eud, eud
	7	60	68	Glob, arom, viri
Waxes (W)	3	81	91	Phenylethyl C22, C24, C26, C28
	5	63	68	Phenylethyl C22, Desmethyl, Eucalyptin
	8	0	6	Benzyl C22, C24, C26, Phenylethyl C24, C25
	11	60	68	Heptadecanone, β-diket C33, total wax
Between-trait-classes				
CM	-	-	-	-
FW	1	64	68	SiderA, Desmethyl, total wax
CT	2	53	58	C hotspot, viri, arom
MW	8	15	20	*Oecophoridae* 1, *Ophelimus* spp., Heptadecanone, β-diket C31
MFT	3	6	14	Grazer 1, Chewer 4, *Phylacteophaga froggatti*, SiderA, neryl
MFW	3	10	15	Chewer 4, *Phylacteophaga froggatti*, Hexadecanal, SiderA

[a] Linkage group.
[b] The beginning, 'start', and end, 'stop', of the QTL hotspot.
[c] The combination of QTL for different traits present in the QTL hotspot. Full names for abbreviated foliar chemistry compounds are in Table 6.2.

No QTL hotspots were detected among individual symptom traits. Three independent between-trait-class QTL hotpots were detected including individual symptoms and foliar chemistry QTL (Fig. 6.1, Table 6.2). QTL for each of the foliar chemistry classes (FPCs, terpenes and waxes) were present in at least one of the hotpots. A QTL for the FPC sideroxylonal A was detected in a hotspot on LG3 along with a QTL for the terpene neryl acetate and QTL for the individual symptoms Grazer 1 and Chewer 4. This hotspot overlapped with another hotspot on LG3 also containing the QTL for sideroxylonal A and Chewer 4 while also containing QTL for the cuticular wax hexadecanal and the insect symptom *Phylacteophaga froggatti*. Only QTL in the hotspot on LG8, including the insect symptoms *Oecophoridae* 1 and *Ophelimus spp.* and the cuticular waxes Heptadecanone and n-Hentriacontane-14, 16-dione (β-diket C31) segregated from the same parent (Table 6.1, Table 6.3).

Additionally, one hotspot was detected among FPCs, three hotspots among terpenes and four hotspots among waxes from the updated and previously reported QTL for foliar chemistry (Fig. 6.1, Table 6.2). The FPC hotspot on LG6 coincides with a hotspot among terpenes consisting of all quantified sesquiterpenes. However, the collapsed hotpots did not significantly co-locate with one another. Despite this, all QTL in this hotspot containing FPCs and terpenes segregated solely from the female parent (Table 6.3). The co-location of QTL for the sesquiterpenes on L6 was first reported in O'Reilly-Wapstra *et al.* (2011) except for the QTL for bicyclogermacrene, which was only detected with the updated map. The two other detected hotspots for terpenes also consisted of only the sesquiterpenes (Fig. 6.1, Table 6.2), with three of the six quantified sesquiterpenes represented in the hotspot on LG7 all segregating solely from the male parent, and four of six on LG2 all segregating solely from the female parent (Table 6.3). None of the QTL in either of these two hotspots were previously detected. The QTL within significant hotspots among waxes presented here (Fig. 6.1, Table 6.2) were first reported to co-locate (peak values within 3cM of one another) in Gosney *et al.* (2016).

Table 6.3. Putative quantitative trait loci (QTL) for formylated phloroglucinols (FPC), terpenes, and waxes in the juvenile foliage of *Eucalyptus globulus* present within QTL hotspots.

Compound[a]	Abbreviation[b]	LG[c]	Nearest Marker[d]	Map pos (cM)	Peak LOD[e]	% exp[f]	Seg.[g]
FPC							
Macrocarpal G	MacG	6	571558	125.1	14.4***	41.6	F
Sideroxylonal A (log)	SiderA	1	565878	64.4	7.9***	8.0	M
		3	644451	10.7	5.1**	4.4	F
		6	571558	125.1	19.6***	27.3	F
Terpenes							
Monoterpenes							
Neryl acetate (log)	neryl	3	Emb115	9.4	4.1	10.2	F
Sesquiterpenes							
Alloaromadendrene (log)	allo	6	571558	124.2	12.6***	41.3	F
Aromadendrene (log)	arom	2	p03b06	57.2	8.0***	8	B
		6	571558	125.1	49.8***	49.8	F
		7	CSRg61	47.9	10.7***	10.7	M
Bicyclogermacrene	bicy	6	571558	124.2	28.8***	28.8	F
β-eudesmol	B-eud	2	573524	12.0	13.1***	3.9	F
		6	571558	125.1	66.7***	82.7	F
Eudesmyl acetate	eud	2	573524	12.0	11.3***	3.7	F
		6	571558	125.1	66.0***	89.4	F
Globulol (log)	glob	2	573524	12.0	9.3***	10.6	F
		6	571558	125.1	25.7***	41.6	F
		7	CSRg61	47.9	7.4***	7.7	M
Viridiflorol	viri	2	p03b06	55.4	4.1	7.6	F
		2	573524	12.0	3.9	7.9	M
		6	571558	125.1	17.3***	42.8	F
		7	CSRg61	48.9	3.8	6.9	M
Waxes							
Aliphatic esters							
Benzyl n-docosanoate (C22)	Benzyl C22	8	599919	1.0	6.1*	19.8	M
Benzyl n-tetracosanoate (C24)	Benzyl C24	8	599919	1.0	16.0***	38.4	M
Benzyl n-hexacosanoate (C26)	Benzyl C26	8	599919	1.0	11.0***	37.1	M
Phenylethyl n-docosanoate (C22)	Phenylethyl C22	3	569486	82.7	10.0***	24.5	B
		5	p02b02	63.8	4.1	8.9	F

Continued next page…

Compound[a]	Abbreviation[b]	LG[c]	Nearest Marker[d]	Map pos (cM)	Peak LOD[e]	% exp[f]	Seg.[g]
Phenylethyl n-tetracosanoate (C24)	Phenylethyl C24	3	p01b01	86.0	6.6**	17.8	B
		8	599919	1.0	8.1***	23.5	M
Phenylethyl n-pentacosanoate (C25)	Phenylethyl C25	8	599919	1.0	3.1	12.1	M
Phenylethyl n-hexacosanoate (C26)	Phenylethyl C26	3	p01b01	86.0	9.4***	29.5	B
Phenylethyl n-octacosanoate (C28)	Phenylethyl C28	3	p01b01	86.0	6.2*	20.3	B
Aliphatic β-diketones							
n-Hentriacontane-14, 16-dione (C31)	B-diket C31	8	503660	19.5	10.6***	30.8	M
n-Tritriacontane-16, 18-dione (C33)	B-diket C33	11	571276	64.2	3.4	9.2	B
Ketones							
Heptadecanone	Heptadecanone	8	566660	15.2	4.0*	11.4	M
		11	571397	63.6	3.5	9.8	B
Aldehydes							
Hexadecanal	Hexadecanal	3	571717	15.0	4.5*	13.5	B
Total Wax	Total wax	1	Emb12	68.1	3.2	7.6	
Flavonoids							
Desmethyl eucalyptin	Desmethyl	1	565878	64.4	9.8***	18.2	M
		5	p02b02	63.8	5.1**	8.4	-
Eucalyptin	Eucalyptin	5	571218	66.3	3.8	8.1	-

[a] Putative QTL for FPCs and terpenes are based on new analyses using the phenotypic data from the original publication and the latest linkage map (see methods for details). The QTL for the waxes were originally published in Gosney *et al.* (2016). Normality transformations for FPC and terpene compounds are presented in parentheses next to the compound name.

[b] Abbreviated compound names used in subsequent figures and tables.

[c] Linkage group

[d] Marker names beginning with Emb or CRC are microsatellites, those named p#b# are AFLP, and the remainder are DArT.

[e] Peak LOD score for each QTL. Genome-wide significance is indicated by: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. The remainder were significant at the suggestive level (chromosome-wide type I error rate <0.05).

[f] The proportion of phenotypic variation between genotype means explained by each QTL.

[g] Whether the QTL segregated only from the male parent (M), the female parent (F) or from both (B).

6.4 Discussion

Numerous QTL for community-level traits, including abundance, richness, evenness and community compositional change, were detected suggesting that fine-scale genetic variation, such as individual QTL, have extended effects in forest trees. Given the noisy nature of community data, the clonal repeatability and effect sizes we report are relatively high for an ecological study. On average, main effects in ecological studies explain less than 6% of the total variation in traits of interest (Møller and Jennions 2002), while in this study QTL effects explained an average of 13% of the variation between genotype means in community-level traits. In *E. globulus*, previous arthropod and fungal community-level studies of provenance variation have reported total extended genetic effects sizes (provenance and family within provenance) averaging 6.4% (Chapter 5). In regard to QTL associated with community compositional change (NMDS), we found that independent directions of compositional change (ie. NMDS1, NMDS2 and NMDS3) have independent genetic control. These QTL were also spread throughout the genome, indicating that community compositional change is likely responding to a wide array of genes. Two of the QTL for community compositional change, NMDS1 on chromosome 2 and NMDS2 on chromosome 9, significantly co-located with independent QTL for total symptom abundance. These co-locations may be due to linkage among loci affecting both these traits. Alternatively, individual locus with pleiotropic effects on community-level traits may underlie one or both co-locations, consistent with the common direction of segregation of the QTL within both hotspots.

All QTL affecting community-level traits in this study are independent of QTL affecting individual community members (symptoms), and are thus emergent genetic effects. Such effects are emergent as they are not predictable based on a knowledge of the host genetic control of the abundance of individual community members (Brown *et al.* 2001; Mayr 1982). Our findings therefore support those of previous studies in that species richness can be viewed as an emergent effect (Brown *et al.* 2001) and provides evidence for a genetic basis of such emergence in forest trees. Additionally, regions of the genome impacting on total abundance are not the same as those controlling the most abundant community members. A consequence of using a symptom-based approach to assess the dependent canopy community is that the few dominant symptoms may mask the impact of less dominant and/or rare symptoms and may have a significantly greater impact on total community abundance. For this reason, it is unexpected that not one of the three identified QTL for total

abundance significantly co-located with any of the five QTL identified for the three dominant symptoms which accounted for 79% of the total abundance on an average tree. None of the QTL identified for the three dominant symptoms significantly co-located with another and all were found on separate chromosomes, indicating the possibility of separate genetic control for the susceptibility to major pest organisms.

At the individual symptom level, our results are consistent with previous findings of QTL for herbivore damage in two forest tree systems, *Populus* and *Salix*, in which QTL for individual damage types rarely co-located with one another (DeWoody *et al.* 2013; Rönnberg-Wästljung *et al.* 2006). In *Populus*, the single case of co-locating QTL was underlain by genes involved in the biosynthetic pathways of known defensive compounds in plants (DeWoody *et al.* 2013). Of the 45 identified QTL for individual symptoms in the present study, no two QTL were found to significantly co-locate. Instead, the three significant hotspots containing one or more QTL for an individual symptom were dependent upon the presence of QTL for foliar chemistry. Of the foliar chemistry QTL significantly co-locating with QTL for one or more individual symptoms, the FPC sideroxylonal A, as well as the cuticular wax Heptadecanone and n-Hentriacontane-14, 16-dione (β-diket C31) have shown previous evidence of bioactivity. In *E. grandis*, the concentration of sideroxylonal A significantly influenced crown damage levels by the leaf chewer *Paropsis atomaria* however, the effect size appeared to be negligible (Henery *et al.* 2008). Despite the small effect size in *E. grandis*, the co-location of QTL for sideroxylonal A with Chewer 4 in the present study provides evidence for a genetic-based influence of a major FPC on leaf chewer abundance in eucalypts. In *E. globulus*, genetic-based variation, with signals of adaptive variation, in the concentration of the cuticular wax Heptadecanone and n-Hentriacontane-14, 16-dione (β-diket C31) has been shown to significantly influence the composition of the dependent canopy community (Chapter 5; Gosney *et al.* 2016). While no QTL for Heptadecanone and n-Hentriacontane-14, 16-dione (β-diket C31) were shown to co-locate with community composition in the present study, the significant hotspot which included QTL for β-diket C31 did include two individual symptoms from distinct damage type guilds. Regardless of the co-locations of QTL for sideroxylonal A and β-diket C31 with individual symptoms, the significance of these hotspots was dependent on the presence of additional compounds with no previous evidence of bioactivity. In a

few cases, these hotspots contained multiple chemical classes, indicating the likely complex network of factors underlying extended genetic effects.

The only QTL for foliar compounds to significantly co-locate with community-level QTL were those for two foliar terpenes; viridiflorol and aromadendrene for which no previous reports of arthropod and fungal bioactivity in *E. globulus* were found. However, in another *Myrtaceae* species, *Melaleuca quinquenervia*, intraspecific variation in concentrations of virdiflorol was under strong genetic control (Padovan *et al.* 2010) and appeared to influence an array of biotic interactions, including oviposition choice of the psyllid *Boreioglycaspis melaleuca* (Wheeler and Ordung 2005) and the feeding and larval development of the weevil *Oxyops vitiosa* (Wheeler 2006). In the case of aromadendrene, this compound has been reported to have antimicrobial properties in *E. globulus* (Mulyaningsih *et al.* 2010), and it is used in the synthesis of insect pheromones for insect trapping (Lamers *et al.* 2003). However, aromadendrene has not been reported to influence arthropod and fungal organisms in *E. globulus*. Additionally, the directions of segregation of the QTL for the community-level traits and foliar chemicals were from different parents in the present study, arguing against pleiotropy and a causal link. Overall, a large component of the genetic-based variation in foliar chemistry appears to have no extended effects. Hotspots containing QTL for numerous chemical compounds, such as the hotpot containing QTL for five cuticular wax compounds on chromosome 8 and the shared hotspot containing QTL for seven terpene compounds and both FPCs on chromosome 6, showed no significant co-locations with individual symptom or community-level trait QTL. The lack of co-locations with community traits for these hotspots in the mapping family is notable as combined these chemical hotspots contain QTL for six foliar compounds (β-eudesmol, eudesmyl acetate, alloaromadendrene, bicyclogermacrene, benzyl n-tertacosanoate C24 and benzyl n-hexacosanoate C26) shown to significantly correlate with arthropod and fungal community variation among provenances of *E. globulus* (Chapter 5). In addition, one of the FPCs has been significantly associated with dependent community variation among provenances (Barbour *et al.* 2009c). This lack of co-location with reported bioactive compounds in *E. globulus* could result from factors including the suggestion that provenance-level correlations could be due to disequilibrium across the broader gene pool (Slatkin 2008) and/or the magnitude of the chemical variation in the mapping family is not sufficient to initiate an extended effect. Such emergent community effects could also be a result of

epistatic interactions among foliar compound QTL. For example, epistatic interactions among QTL regulating leaf aliphatic glucosinolate concentrations in *Arabidopsis thaliana* have been implicated in the resistance to the feeding of the generalist insect herbivore *Trichoplusia ni* (Kliebenstein *et al*. 2002).

In conclusion, numerous QTL were detected for individual symptom and community-level traits indicating that community traits respond to many different facets of the genetic-based variation across the genome. The peak locations of QTL for community-level traits were independent of the QTL for individual symptoms comprising the community, arguing these are emergent genetic effects. Further, despite several co-location hotspots, there was no evidence for a causal link between community-level QTL and QTL for foliar chemistry. QTL hotspots for foliar compounds with previous evidence of bioactivity in *E. globulus* had no detectable influence on community traits. This study highlights the complexity underlying phenotypic variation in community traits, while providing evidence of genetic-based emergence of community-level effects.

6.5 Acknowledgments

We thank Anthony Mann and Dave McElwee for help in collection of the samples involved in the study and Forestry Tasmania and Forico Pty Limited for access to the trials. This study is part of a PhD undertaken by Benjamin Gosney, supported by an International Postgraduate Scholarship provided by the University of Tasmania and funded by the Australian Government. Data collection was funded by Australian Research Council Discovery grants (DP140102552 and DP0773686) and a Linkage grant (LP120200380 in partnership with Greening Australia).

Chapter 7: Discussion

Four key findings emerged from this thesis. First, significant genetic-based provenance effects on dependent canopy community responses are shown in three independent and important eucalypt systems. Second, while genetic-based provenance effects on canopy communities are relatively small in magnitude compared to site and year effects, broad trends in genetic-based community variation were associated with reported patterns of adaptive variation in the focal tree species and were detected across sites and years. Third, evidence linking foliar chemistry as potential mechanistic drivers of provenance differences in canopy communities is obtained, with chemistry accounting for a substantial proportion of the extended genetic effects of eucalypt provenances. Fourth, while QTL were detected for community-level responses in *E. globulus*, none of the individual symptoms comprising the community nor the chemical compounds associated with provenance differences in the canopy community were associated with these genomic regions. These findings are discussed below in terms of their relevance and the original issues raised.

7.1 Importance of community genetic effects in eucalypts

Genetic-based provenance effects on the dependent arthropod and fungal canopy community were found to be significant in three independent and important eucalypt systems. Tack and Roslin (2011) suggested that the impact of forest tree genetic variation on dependent communities is system, scale, and context dependent, with genetic effects playing a minor role at large spatial scales. Few studies have examined this issue in forest trees using multiple common garden trials and those that have are rarely repeated over multiple years. However, these studies show a minor impact of genetic effects across multiple common garden trials with the narrowleaf cottonwood *Populus angustifolia* (Busby *et al.* 2014), the silver birch *Betula pendula* (Silfver et al. 2014) and the English oak *Quercus robur* (Tack and Roslin 2011) showing variation in the dependent community between common garden trial sites accounting for up to 4 times greater than that of genetic effects. The only study to repeat the experiment over multiple years found little differences in the dependent communities among years (Busby *et al.* 2014). The findings in this thesis show that the tree genus *Eucalyptus* is no exception. Indeed, community genetic effects in eucalypts

were relatively small, with provenance differences accounting for an average of 9.5% (*E. morrisbyi* – single site), 5.6% (*E. globulus* – across sites) and 2.8% (*E. pauciflora* – across years and sites) of the variation in dependent arthropod and fungal community composition with site and year accounting for up to 6 times the variation of provenance effects. Additionally, QTL effects in *E. globulus* explained an average of 12.5% of the total variation in the dependent community composition. The discrepancy between the findings in this thesis and the minimal impact of year in *P. angustifolia* highlights the variability in effects such as year in community and ecological studies. Indeed, infestations of an organism/organisms in a specific year may have substantially affected the degree to which genetic effects were expressed in *E. pauciflora*. Due to the lack of community genetics studies examining spatial and temporal effects simultaneously and the findings in this thesis, it is recommended that future studies of community genetics in forest trees be based on long-term assessments of multiple common garden trials across a range of environments associated with the species of interest (Tack *et al.* 2010). Ideally, common garden trials would be established across the geographic range of the tree species with each provenance location having a represented common garden trial. However, such a costly and time-consuming endeavor is likely impractical for long-lived forest trees systems and thus maximizing the environmental variation between a few trial sites may be a plausible alternative.

7.2 Provenance variation and potential biotic impacts of translocation

This thesis supports the suggestion that community genetic effects associated with provenance differences within forest tree species are small in comparison to effects such as site and year. However, findings also indicate that focusing solely on the magnitude of the genetic effect is likely to ignore important underlying spatial and adaptive trends in provenance variation. This thesis has shown that broad community genetic trends associated with adaptive variation among provenances of eucalypts are evident and appear stable across sites and years. There have been few community genetic studies of inter-provenance variation within species, yet for foundation species such as trees the extended effects of provenance variation to the community and ecosystem levels has important implication for provenance choice in ecological restoration, conservation strategies, and management responses to global climate change such as assisted migration and assisted gene flow. Thus, the findings of this thesis support recent concerns of the potential biotic consequences of provenance translocations (Bucharova 2016), which are being increasingly promoted in

commercial (Gray *et al.* 2016), conservation (Aitken and Whitlock 2013) and restoration (Breed *et al.* 2013; Prober *et al.* 2016) practices. While the extent to which such community genetic effects feedback to impact population fitness is unclear, translocations of non-local provenances could potentially alter the ecological or evolutionary trajectory of local biotic communities.

While an underlying trend of provenance variation was previously reported in *E. globulus* (Barbour *et al.* 2009c), which coincided with the latitudinal trend detected here, this thesis provide evidence of stability in such trends across sites and years. Additionally, this thesis showed non-local mainland provenances supported more dependent organisms than the Tasmanian provenances of *E. globulus,* which could be due to several factors including the lack of adaptation of non-local provenances to resist colonization by the local biotic community or an adverse interaction with a dominant community member such as *Teratosphaeria* sp. 1, which could directly or indirectly reduce suitable habitat for other organisms (Borzak *et al.* 2015). Some dependent organisms have deleterious fitness effects on the focal trees (Jordan *et al.* 2002; Milgate *et al.* 2005a), indicating the possibility that non-local provenances could perform poorly in large-scale plantings. This was also evident in *E. pauciflora* where provenances from a higher home-site altitude than the common garden sites exhibited a greater proportion of overall herbivore damage and fewer causal organisms, while the provenances from a lower home-site altitude showed the least herbivore damage and a greater number of causal organisms. These findings argue that provenance translocations in commercial, conservation and restoration practices will have a significant impact on the biotic community. However, the idea of what is 'local' appears to be driven by broad scale trends of adaptation rather than geographic proximity of provenances. For example, in regard to the impact on the biotic community the mid-altitudinal provenances of *E. pauciflora* may be considered for translocation to sites within this mid-altitudinal range regardless of the provenance home-site geographic distance from the planting site. These findings indicate a need for further research into what difference between provenances initiates a change in the trajectory of the colonizing biotic community begin to change, to determine what can be considered 'local'. Indeed, 'local' from a genetics perspective may differ from that of a community genetics perspective. However, in the case of the European aspen *Populus tremula*, it all seemed consistent with fewer herbivores observed on local trees compared to trees from more distant localities (Bernhardsson *et al.* 2013). Nevertheless, further research on the effect underlying trends

in host provenance adaptation on the dependent biotic community is still necessary. How adaptive variation is driving community responses (i.e. the potential mechanisms) and the stability of these trends over the course of many years/decades is still poorly understood.

7.3 Foliar chemistry as drivers of provenance variation in community responses

Evidence was detected suggesting that adaptive variation in cuticular wax compounds, in part, contribute to provenance differences in community responses of *E. globulus*. While cuticular waxes have long been implicated in influencing insect herbivory (Edwards 1982; Jones *et al.* 2002), this thesis suggests a phenotypic and genetic-based community-level influence. The four cuticular waxes linked as potential mechanisms driving genetic-based differences in the dependent community of *E. globulus* were also those showing signals of diversifying selection in this thesis, supporting the potential for extended consequences of adaptive variation to the biotic community (Bucharova 2016). While genetic-based adaptive variation in phenotypic traits along environmental gradients have been reported in many forest trees (Bresson *et al.* 2011; Morgenstern 2011; Mousseau *et al.* 2000; O'Reilly-Wapstra *et al.* 2013b), few studies have examined whether these traits drive or are driven by the dependent biotic community. Many studies have noted the importance of identifying the traits driving community responses (Crutsinger 2016; Hersch-Green *et al.* 2011; Whitham *et al.* 2006), however, they have been largely unexplored. Of the potential traits driving genetic-based variation in community responses, phytochemistry is perhaps the most widely examined with studies showing significant associations with genetic-based variation in dependent community responses (Barbour *et al.* 2009c; Whitham *et al.* 2006). This thesis partitions the community variation associated with foliar chemistry in forest trees, allowing for assessment of the magnitude of their phenotypic and genetic-based influence. While the findings in this thesis provide a significant step forward in explaining how genetic-based variation may influence community responses, there is still much to explore. Only two foliar chemical classes, the terpenes and the waxes, were examined in this thesis, accounting for 8.6% of the phenotypic and 34.3% of the genetic-based variation in the dependent canopy community, leaving a large portion of the total variation still unexplained. Given the substantial influence of the two foliar chemical classes examined in this thesis, further investigation of other foliar chemistry implicated in bioactivity, such as condensed tannins (Barbour *et al.* 2009c; Cornelissen and Stiling 2006; Forkner *et al.* 2004; Whitham *et al.* 2006; Yarnes *et al.* 2008) and formylated phloroglucinol compounds

(Barbour *et al.* 2009c; Henery *et al.* 2008; Matsuki *et al.* 2011) is warranted. However, it has been recently argued that too much focus has been given to the implication of phytochemistry in community genetics research, ignoring the importance of other potential drivers, including plant physiology, morphology and phenology (Crutsinger 2016). For example, there is evidence that genetic variation in morphological traits, such as plant growth, may have a stronger impact on dependent community responses than that of chemical variation (Robinson *et al.* 2012). Ideally, it is recommended that future community genetics studies in forest trees assess as many traits showing phenotypic variation as possible at the same time as community assessment to provide a more comprehensive view of the drivers of phenotypic and genetic-based variation in community responses.

7.4 The emergent community-level QTL of *E. globulus*

This thesis provides evidence of QTL for community-level responses in the forest tree *E. globulus*, while also providing evidence of genetic-based emergence of community-level effects. Two previous studies of potential community-level QTL in other forest tree systems focused on QTL detection for independent guild assemblage abundances (DeWoody *et al.* 2013; Rönnberg-Wästljung *et al.* 2006), while this thesis detected QTL for overall community-level parameters and composition which include numerous functional guilds. This thesis shows that fine-scale genetic variation in forest trees can have extended genetic effects with the detection of numerous QTL for community-level traits in *E. globulus*, including abundance, richness, evenness and community composition. The emergence of the community-level QTL suggests an incongruity between broad- and fine-scale genetic effects, with no detectable mechanistic link of foliar chemistry at the QTL level, despite chemistry accounting for 34% of the provenance variation in the dependent community. This could be due to a limitation in the study in which community assessment was done on adult foliage, while the foliar chemistry was previously done on the juvenile foliage of the *E. globulus* F_2 mapping family. Thus, it is suggested that this be further investigated with assessment of the foliar chemistry at the same time as the community assessment to eliminate the potential that different QTL influence foliar chemistry between ontogenetic stages. However, this does not account for the emergence of the community-level QTL in regard to the QTL for individual community members. While the findings in this thesis provide an increased understanding of the genetic-based provenance effects on dependent biotic communities, it also

highlights the limited understanding and need for further research of how the underlying regions of the genome are expressing these provenance differences.

7.5 Management implications

A community genetics approach has been proposed as a more realistic strategy in species management as understanding the extended genetic effects of management species considers more of the factors involved and, thus, is less likely to result in management errors and unintended effects (Whitham *et al.* 2010). Indeed, the potential extended genetic effects on biotic communities of provenance translocations in commercial, conservation and restoration practices of eucalypts is evident from the findings of this thesis. All three *Eucalyptus* species showed significant provenance variation in their dependent arthropod and fungal communities despite the substantial influence of site and year effects. These extended genetic effects have been shown to influence organism abundance, richness and overall composition, with apparent underlying trends associated with spatial and adaptive variation within the species detected across sites and years. Potential implications and recommendations in regard to provenance translocations of the species studied in this thesis are discussed below.

In *E. morrisbyi*, the dramatic decline of the larger Calverts Hill population (of the 2 main populations of *E. morrisbyi*) over that last decade has renewed concern into potential conservation strategies for the species. This decline is believed to be due to the increased susceptibility of the Calverts Hill population to insect herbivory, which was noted with a greater abundance of symptoms on the Calverts Hill population compared to the Risdon Hill population in this thesis. The Calverts Hill population is also more susceptible to browsing by the major marsupial herbivore *Trichosurus vulpecula* (Mann *et al.* 2012), which may also be contributing to the decline of the population. As a potential management strategy to the decline of the Calverts Hill population, translocation of seed from the Risdon Hill population to help restore the fitness of the Calverts Hill population is being discussed (Jones *et al.* 2016). While this thesis supports the populations being maintained as distinct management units, the potential that such translocation may benefit the survivability *E. morrisbyi* at Calverts Hill population through introduction of a population to which the current biotic community may not be adapted cannot be ignored. Indeed, when provided germplasm location is monitored such strategies can be readily tested with the options of removal

of non-local germplasm prior to flowering or if the risk of the local germplasm abates. Nevertheless, there are other emerging risks which need close monitoring. The populations have recently been shown to differ markedly in their susceptibility to myrtle rust, an exotic pathogen to which the Myrtaceae are susceptible (Potts *et al.* 2016). The Risdon Hills germplasm is much more susceptible than that from the Calverts Hill population, thus, its introduction into the Calverts Hill population could increase population-level disease risk if the pathogen was to spread to native forests in Tasmania.

In the case of *E. pauciflora*, large-scale restoration strategies have been proposed in drought prone environments of Tasmania exhibiting large-scale tree decline (Bailey 2013). Numerous restoration sites have already been established (including the trials assessed in this thesis), and with the potential need to use non-local provenances to account for future climates (Harrison *et al.* 2017), it has been important to understand the potential extended consequences of provenance translocations. The apparent altitudinal cline associated with the dependent community of *E. pauciflora* shown in this thesis suggests that translocations of non-local provenances within the relative altitudinal range of the restoration sites (i.e. high, mid and low altitude) will have little impact on the trajectory of the biotic community. However, these provenances may not possess the traits of interest (i.e. drought resistance) necessary for survivability at the restoration sites (O'Brien *et al.* 2007), regardless of whether the biotic community may be adapted to these provenances (Evans *et al.* 2008; Whitham *et al.* 2010). While this thesis suggests that the translocation of non-local provenances will affect the trajectory of the colonizing community, and there is some predictability to this (e.g. altitude), the long-term consequences of such community genetic differences can only be speculated, particularly in the face of the direct effects of climate and other environmental changes. However, in terms of overall rather than canopy-level biodiversity, the demonstration of community genetic effects at the provenance level would argue that plantings with mixes of provenances will develop more biodiverse communities. Numerous studies have indicated that genetically diverse plantings increase dependent community biodiversity (Crutsinger *et al.* 2006; Johnson *et al.* 2006; Tovar-Sanchez and Oyama 2006; Whitham *et al.* 2010; Wimp *et al.* 2004), and that further research is required to examine these effects. Similarly, with the restoration sites sampled at a relatively young age in this thesis and for only a specific component of the dependent community, further research is required to test whether

community genetic effects become more prominent with age and extend to other types of communities both above and below ground.

In *E. globulus*, translocation of seed has long been a practiced due to the commercial use of the species worldwide. While the species is native to southeastern Australia and Tasmania, the species has been planted for forestry use globally (Potts *et al.* 2004). Due to the global forestry plantings, the effects of *E. globulus* as a species on associated biotic communities has been documented in several countries (Ferreira *et al.* 2006; Garrison 1998; Paine *et al.* 2011). However, it is now evident that such extended impacts of *E. globulus* plantings may in part vary dependent on tree genotype, which is predictable to some extent at the sub-race level (Barbour *et al.* 2009c; present thesis). Certainly, in an Australian environment where plantings occur in close proximity to native forests and are rapidly colonized by native, often generalist, arthropods and fungi, the biotic communities that develop in the planted forests will vary dependent on the broad-region of origin of the germplasm. Such translocations could potentially affect pest management regimes (Garnas *et al.* 2012), as well as alter the surrounding native forest biotic community with flow-over effects from the planted forests (Jairus *et al.* 2011; Murray *et al.* 2010; Ratnam *et al.* 2014). However, 'local' provenances from a community genetics perspective appears to be provenances within the same lineage of *E. globulus* (i.e. mainland Australian lineage, Western Tasmanian lineage, and eastern Tasmanian lineage), which were evident in the independent latitudinal and longitudinal gradients of the cuticular wax compounds significantly influencing the dependent canopy community. The extent to which the canopy community itself feedbacks to affect tree vigour and negatively impact commercial plantings is unclear in the present case. There is evidence under high levels of damage, specific community members such as the fungus *Teratosphaeria* (Milgate et al. 2005a) and the canopy defoliating sawflies *Perga affinis* (Jordan et al. 2002) can have adverse effects on the growth of genetically susceptible trees. However, in the case of this disease, the mainland sub-races are more resistant in both juvenile (Hamilton *et al.* 2013) and adult foliage (present thesis), but more susceptible to sawfly damage (Jordan *et al.* 2002)

7.5 Conclusion

The findings in this thesis provide further support that forest tree genetic variation can have extended effects on biotic communities. Further, evidence was presented indicating that adaptive

variation in phenotypic traits, such as foliar chemistry, can contribute to a substantial proportion of the genetic-based provenance variation in canopy community responses, which appears to be consistent over sites and years. Thus, it is argued that translocation of non-local provenances of forest trees in commercial, conservation and restoration practices can have a detectable impact on the biotic community, potentially altering the ecological and evolutionary trajectory of the community. However, the degree to which a provenance can be considered 'local' in terms of the impact on the biotic community garners further investigation. Additionally, while provenance effects on canopy communities in eucalypts is evident, the apparent emergent community-level QTL of *E. globulus* highlights the complexity underlying both phenotypic and genetic-based variation in dependent canopy community responses of forest trees.

References

Abbott I, Wills A (2001) Biodiversity of canopy arthropods in jarrah forest of south-west western Australia: review of ecological theory and conservation management. *Pacific Conservation Biology* **7**, 101-117.

Abdala-Roberts L, Mooney K (2014) Ecological and evolutionary consequences of plant genotype diversity in a tri-trophic system. *Ecology* **95**, 2879-2893.

Agrawal A (2005) Natural selection on common milkweed (*Asclepias syriaca*) by a community of specialized insect herbivores. *Evolutionary Ecology Research* **7**, 651-667.

Aitken S, Whitlock M (2013) Assisted gene flow to facilitate local adaptation to climate change. *Annual Review of Ecology, Evolution, and Systematics* **44**, 367-388.

Al-Abdallat A, Al-Debei H, Ayad J, Hasan S (2014) Over-expression of SlSHN1 gene improves drought tolerance by increasing cuticular wax accumulation in tomato. *International Journal of Molecular Sciences* **15**, 19499-19515.

Anderson M, Gorley R, Clarke K (2008) 'PERMANOVA+ for PRIMER: guide to software and statistical methods.' (PRIMER-E Ltd.: Plymouth, UK).

Anderson M, Willis T (2003) Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. *Ecology* **84**, 511-525.

Andrew R, Wallis I, Harwood C, Foley W (2010) Genetic and environmental contributions to variaition and population divergence in a broad-specturm foliar defence of *Eucalyptus tricarpa*. *Annals of Botany* **105**, 707-717.

Andrew R, Wallis I, Harwood C, Henson M, Foley W (2007) Heritable variation in the foliar secondary metabolite sideroxylonal in *Eucalyptus* confers cross-resistance to herbivores. *Oecologia* **153**, 891-901.

Axelsson E, Iason G, Julkunen-Tiitto R, Whitham T (2015) Host genetics and environment drive divergent responses of two resource sharing gall-formers on Norway spruce: a common garden analysis. *PloS One* **10**, e0142257.

Bailey J, Schweitzer J, Ubeda F, Zinkgraf M, Fitzpatrick B, O'Reilly-Wapstra J, Rehhill B, LeRoy C, Potts B, Whitham T, Genung M, Fischer D, Pregitzer C, Keith A (2011) From genes to ecosystems: emerging concepts bridging ecological and evolutionary dynamics. In 'Ecological Reviews-The Ecology of Plant Secondary Metabolites: From Genes to

Landscapes'. (Eds G Iason, M Dicke and S Hartley). (Cambridge University Press: Cambridge).

Bailey J, Wooley S, Lindroth R, Whitham T (2006) Importance of species interactions to community heritability: a genetic basis to trophic-level interactions. *Ecology Letters* **9**, 78-85.

Bailey T (2013) Plantings for carbon, biodiversity and restoration in dry rural landscapes. *Australian Forest Grower* **35**, 39.

Baker E (1982) Chemistry and morphology of plant epicuticular waxes. In 'The Plant Cuticle'. (Eds D Cutler, K Alvin and C Price) pp. 139-165. (Academic Press: London).

Bangert R, Allan G, Turek R, Wimp G, Meneses N, Martinsen G, Keim P, Whitham T (2006a) From genes to geography: a genetic similarity rule for arthropod community structure at multiple geographic scales. *Molecular Ecology* **15**, 4215-4228.

Bangert R, Turek R, Rehill B, Wimp G, Schweitzer J, Allan G, Bailey J, Martinsen G, Keim P, Lindroth R, Whitham T (2006b) A genetic similarity rule determines arthropod community structure. *Molecular Ecology* **15**, 1379-1391.

Barbehenn R, Constabel C (2011) Tannins in plant–herbivore interactions. *Phytochemistry* **72**, 1551-1565.

Barber H (1955) Adaptive gene substitutions in Tasmanian eucalypts: I. Genes controlling the development of glaucousness. *Evolution* **9**, 1-14.

Barber H, Jackson W (1957) Natural selection in action in *Eucalyptus*. *Nature* **179**, 1267 - 1269.

Barbour M, Rodriguez-Cabal M, Wu E, Julkunen-Tiitto R, Ritland C, Miscampbell A, Jules E, Crutsinger G (2015) Multiple plant traits shape the genetic basis of herbivore community assembly. *Functional Ecology* **29**, 995-1006.

Barbour R, Baker S, O'Reilly-Wapstra J, Harvest T, Potts B (2009a) A footprint of tree-genetics on the biota of the forest floor. *Oikos* **118**, 1917-1923.

Barbour R, Forster L, Baker S, Steane D, Potts B (2009b) Biodiversity consequences of genetic variation in bark characteristics within a foundation tree species. *Conservation Biology* **23**, 1146-1155.

Barbour R, O'Reilly-Wapstra J, Little D, Jordan G, Steane D, Humphreys J, Bailey J, Whitham T, Potts B (2009c) A geographic mosaic of genetic variation within a foundation tree species and its community-level consequences. *Ecology* **90**, 1762-1772.

Barthlott W, Neinhuis C, Cutler D, Ditsch F, Meusel I, Theisen I, Wilhelmi H (1998) Classification and terminology of plant epicuticular waxes. *Journal of the Linnean Society* **126**, 237-260.

Barton K, Valkama E, Vehviläinen H, Ruohomäki K, Knight T, Koricheva J (2015) Additive and non-additive effects of birch genotypic diversity on arthropod herbivory in a long-term field experiment. *Oikos* **124**, 697-706.

Batish D, Singh H, Kohli R, Kaur S (2008) *Eucalyptus* essential oil as a natural pesticide. *Forest Ecology and Management* **256**, 2166-2174.

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate - A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B-Methodological* **57**, 289-300.

Bennett R, Wallsgrove R (1994) Secondary metabolites in plant defence mechanisms. *New Phytologist* **127**, 617-633.

Bernays E (1981) Plant tannins and insect herbivores: an appraisal. *Ecological Entomology* **6**, 353-360.

Bernhardsson C, Robinson K, Abreu I, Jansson S, Albrectsen B, Ingvarsson P (2013) Geographic structure in metabolome and herbivore community co-occurs with genetic structure in plant defence genes. *Ecology Letters* **16**, 791-798.

Bito D, Novotny V, Burwell C, Nakamura A, Kitching R, Ødegaard F (2011) Predator pressure, herbivore abundance and plant damage along a subtropical altitudinal gradient. *Memoirs of the Queensland Museum* **55**, 451-461.

Blackhall S, Lynch J (1992) '*Eucalyptus morrisbyi* flora Recovery Plan: Management phase.' (Department of Parks, Wildlife and Heritage: Hobart, Tasmania).

Bodnaryk R (1992) Leaf epicuticular wax, an antixenotic factor in *Brassicaceae* that affects the rate and pattern of feeding of flea beetles, *Phyllotreta cruciferae* (Goeze). *Canadian Journal of Plant Science* **72**, 1295-1303.

Boland D, Brooker M, Chippendale G, Hall N, Hyland B, Johnston R, Kleinig D, McDonald M, Turner J (2006) 'Forest trees of Australia.' (CSIRO publishing).

Boland D, Brophy J, House A (1991) *Eucalyptus* leaf oils: use, chemistry, distillation and marketing.

Borisjuk N, Hrmova M, Lopato S (2014) Transcriptional regulation of cuticle biosynthesis. *Biotechnology Advances* **32**, 526-540.

Borzak C, O'Reilly-Wapstra J, Potts B (2015) Direct and indirect effects of marsupial browsing on a foundation tree species. *Oikos* **124**, 515-524.

Borzak C, Potts B, Davies N, O'Reilly-Wapstra J (2014) Population divergence in the ontogenetic trajectories of foliar terpenes of a *Eucalyptus* species. *Annals of Botany* **115**, 159-170.

Bradshaw H, Stettler RF (1995) Molecular genetics of growth and development in populus. IV. Mapping QTLs with large effects on growth, form, and phenology traits in a forest tree. *Genetics* **139**, 963-973.

Breed M, Stead M, Ottewell K, Gardner M, Lowe A (2013) Which provenance and where? Seed sourcing strategies for revegetation in a changing environment. *Conservation Genetics* **14**, 1-10.

Brennan E, Weinbaum S (2001) Effect of epicuticular wax on adhesion of psyllids to glaucous juvenile and glossy adult leaves of *Eucalyptus globulus* Labillardière. *Australian Journal of Entomology* **40**, 270-277.

Bresson C, Vitasse Y, Kremer A, Delzon S (2011) To what extent is altitudinal variation of functional traits driven by genetic adaptation in European oak and beech? *Tree Physiology* **31**, 1164-1174.

Broadhurst L, Lowe A, Coates D, Cunningham S, McDonald M, Vesk P, Yates C (2008) Seed supply for broadscale restoration: maximizing evolutionary potential. *Evolutionary Applications* **1**, 587-597.

Brown J, Ernest S, Parody J, Haskell J (2001) Regulation of diversity: maintenance of species richness in changing environments. *Oecologia* **126**, 321-332.

Brunet S, Jackson F, Hoste H (2008) Effects of sainfoin (*Onobrychis viciifolia*) extract and monomers of condensed tannins on the association of abomasal nematode larvae with fundic explants. *International Journal for Parasitology* **38**, 783-790.

Bucharova A (2016) Assisted migration within species range ignores biotic interactions and lacks evidence. *Restoration Ecology* **25**, 14-18.

Burdon J, Chilvers G (1974) Leaf parasites on altitudinal populations of *Eucalyptus pauciflora* Sieb. ex Spreng. *Australian Journal of Botany* **22**, 265-269.

Busby P, Lamit L, Keith A, Newcombe G, Gehring C, Whitham T, Dirzo R (2015) Genetics-based interactions among plants, pathogens, and herbivores define arthropod community structure. *Ecology* **96**, 1974-1984.

Busby P, Necombe G, Dirzo R, Whitham T (2014) Differentiating genetic and environmental drivers of plant-pathogen community interactions. *Journal of Ecology* **102**, 1300-1309.

Busby P, Newcombe G, Dirzo R, Whitham T (2013) Genetic basis of pathogen community structure for foundation tree species in a common garden and in the wild. *Journal of Ecology* **101**, 867-877.

Buschhaus C, Jetter R (2010) Composition differences between epicuticular and intracuticular wax substructures: How do plants seal their epidermal surfaces? *Journal of Experimental Botany* **62**, 841-853.

Bustos-Segura C, Külheim C, Foley W (2015) Effects of terpene chemotypes of *Melaleuca alternifolia* on two specialist leaf beetles and susceptibility to myrtle rust. *Journal of Chemical Ecology* **41**, 937-947.

Butler J, Freeman J, Vaillancourt R, Potts B, Glen M, Lee D, Pegg G (2016) Evidence for different QTL underlying the immune and hypersensitive responses of *Eucalyptus globulus* to the rust pathogen *Puccinia psidii*. *Tree Genetics & Genomes* **12**, 1-13.

Byrne M (2008) Phylogeny, diversity and evolution of eucalypts. In 'Plant Genome: Biodiversity and Evolution'. Vol. 1. (Eds A Sharma and A Sharma) pp. 303-346. (Science Publishers: Enfield, NH).

Byrne M, Murrell J, Owen J, Williams E, Moran G (1997) Mapping of quantitative trait loci influencing frost tolerance in *Eucalyptus nitens*. *Theoretical and Applied Genetics* **95**, 975-979.

Carnegie A, Keane P, Ades P, Smith I (1994) Variation in susceptibility of *Eucalyptus globulus* provenances to *Mycosphaerella* leaf disease. *Canadian Journal of Forest Research* **24**, 1751-1757.

Carvalho M, Wilf P, Barrios H, Windsor D, Currano E, Labendeira C, Jaramillo C (2014) Insect leaf-chewing damage tracks herbivore richness in modern and ancient forests. *PloS One* **9**, e94950.

Centre for Plant Biodiversity Research, Slee A (2006) 'EUCLID eucalypts of Australia.' (CSIRO Publ.).

Chassot C, Nawrath C, Metraux J (2007) The cuticle: not only a barrier for defence. *Plant Signaling & Behavior* **3**, 142-144.

Close D, Davidson N, Shields C, Wiltshire R (2007) Reflectance and phenolics of green and glaucous leaves of *Eucalyptus urnigera*. *Australian Journal of Botany* **55**, 561-567.

Compson Z, Hungate B, Koch G, Hart S, Maestas J, Adams K, Whitham T, Marks J (2015) Closely related tree species differentially influence the transfer of carbon and nitrogen from leaf litter up the aquatic food web. *Ecosystems* **18**, 186-201.

Compson Z, Hungate B, Whitham T, Meneses N, Busby P, Wojtowicz T, Ford A, Adams K, Marks J (2016) Plant genotype influences aquatic-terrestrial ecosystem linkages through timing and composition of insect emergence. *Ecosphere* **7**.

Cone K, McMullen M, Bi I, Davis G, Yim Y, Gardiner J, Polacco M, Sanchez-Villeda H, Fang Z, Schroeder S (2002) Genetic, physical, and informatics resources for maize. On the road to an integrated map. *Plant Physiology* **130**, 1598-1605.

Cornelissen T, Stiling P (2006) Responses of different herbivore guilds to nutrient addition and natural enemy exclusion. *Ecoscience* **13**, 66-74.

Costa e Silva J, Borralho N, Araujo J, Vaillancourt R, Potts B (2009) Genetic parameters for growth, wood density and pulp yield in *Eucalyptus globulus*. *Tree Genetics & Genomes* **5**, 291–305.

Costa e Silva J, Potts B, Dutkowski G (2006) Genotype by environment interaction for growth of *Eucalyptus globulus* in Australia. *Tree Genetics & Genomes* **2**, 61-75.

Crous P, Braun U, Groenewald J (2007) *Mycosphaerella* is polyphyletic. *Studies in Mycology* **58**, 1-32.

Crutsinger G (2016) A community genetics perspective: opportunities for the coming decade. *New Phytologist* **210**, 65-70.

Crutsinger G, Collins M, Fordyce J, Gompert Z, Nice C, Sanders N (2006) Plant genotypic diversity predicts community structure and governs an ecosystem process. *Science* **313**, 966-968.

CycSoftware (2009) 'A package for the computer generation of experimental designs.' Version 4.0 edn. (CycSoftware Ltd: Hamilton, New Zealand).

DeWoody J, Viger M, Lakatos F, Tuba K, Taylor G, Smulders M (2013) Insight into the genetic components of community genetics: QTL mapping of insect association in a fast-growing forest tree. *PloS One* **8**, e79925.

Dodd R, Poveda M (2003) Environmental gradients and population divergence contribute to variation in cuticular wax composition in *Juniperus communis*. *Biochemical Systematics and Ecology* **31**, 1257–1270.

Dodd R, Rafii Z, Power A (1998) Ecotypic adaptation in *Austrocedrus chilensis* in cuticular hydrocarbon composition. *New Phytologist* **138**, 699-708.

Dungey H, Potts B, Carnegie A, Ades P (1997) *Mycosphaerella* leaf disease: genetic variation in damage to *Eucalyptus nitens*, *Eucalyptus globulus*, and their F1 hybrid. *Canadian Journal of Forest Research* **27**, 750-759.

Dungey H, Potts B, Whitham T, Li H (2000) Plant genetics affects arthropod community richness and composition: Evidence from a synthetic eucalypt hybrid population. *Evolution* **54**, 1938-1946.

Dutkowski G, Potts B (2012) Genetic variation in the susceptibility of *Eucalyptus globulus* to drought damage. *Tree Genetics & Genomes* **8**, 757-773.

Dutkowski GW, Potts BM (1999) Geographic patterns of genetic variation in *Eucalyptus globulus ssp globulus* and a revised racial classification. *Australian Journal of Botany* **47**, 237-263.

Edelaar P, Björklund M (2011) If FST does not measure neutral genetic differentiation, then comparing it with QST is misleading. Or is it? *Molecular Ecology* **20**, 1805-1812.

Edwards P (1982) Do waxes on juvenile *Eucalyptus* leaves provide protection from grazing insects? *Australian Journal of Ecology* **7**, 347–352.

Edwards P, Wanjura W, Brown W (1993) Selective herbivory by Christmas beetles in response to intraspecific variation in *Eucalyptus* terpenoids. *Oecologia* **95**, 551-557.

Edwards P, Wanjura W, Brown W, Dearn J (1990) Mosaic resistance in plants. *Nature London* **347**.

Eigenbrode S (2004) The effects of plant epicuticular waxy blooms on attachment and effectiveness of predatory insects. *Arthropod Structure and Development* **33**, 91-102.

Eigenbrode S, Espelie K (1995) Effects of plant epicuticular lipids on insect herbivores. *Annual Review of Entomology* **40**, 171-194.

Eigenbrode S, Jetter R (2002) Attachment to plant surface waxes by an insect predator. *Integrative and Comparative Biology* **42**, 1091-1099.

Ellison A, Bank M, Clinton B, Colburn E, Elliott K, Ford C, Foster D, Kloeppel B, Knoepp J, Lovett G, Mohan J, Orwig D, Rodenhouse N, Sobczak W, Stinson K, Stone J, Swan C, Thompson J, Von Holle B, Webster J (2005) Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment* **3**, 479-486.

Endler J, Mazer S, Williams M, Sandoval C, Ferren W (2010) 'Problems associated with the introduction of non-native genotypes on NRS reserves.' Available at http://www.ucnrs.org/staff/admin_handbook/APP-18ABCD-Use-Application/Non-Native-Genotype.pdf [Verified 2 Feb 2017]

Ennis D, Despland E, Chen F, Forgione P, Bauce E (2016) Spruce budworm feeding and oviposition are stimulated by monoterpenes in white spruce epicuticular waxes. *Insect Science* **24**, 1-8.

Ernest S, Brown J (2001) Homeostasis and compensation: the role of species and resources in ecosystem stability. *Ecology* **82**, 2118-2132.

Evans L, Allan G, Shuster S, Woolbright S, Whitham T (2008) Tree hybridization and genotypic variation drive cryptic speciation of a specialist mite herbivore. *Evolution* **62**, 3027-3040.

Falcone Ferreyra M, Rius S, Casati P (2012) Flavonoids: biosynthesis, biological functions, and biotechnological applications. *Frontiers in Plant Science* **3**, 222.

Ferreira V, Elosegi A, Gulis V, Pozo J, Graça M (2006) *Eucalyptus* plantations affect fungal communities associated with leaf-litter decomposition in Iberian streams. *Archiv für Hydrobiologie* **166**, 467-490.

Fletcher D, Underwood A (2002) How to cope with negative estimates of components of variance in ecological field studies. *Journal of Experimental Marine Biology and Ecology* **273**, 89-95.

Floate K, Kearsley M, Whitham T (1993) Elevated herbivory in plant hybrid zones - *Chrysomela*-confluens, *Populus* and phenological sinks. *Ecology* **74**, 2056-2065.

Forkner R, Marquis R, Lill J (2004) Feeny revisited: condensed tannins as anti-herbivore defences in leaf-chewing herbivore communities of *Quercus*. *Ecological Entomology* **29**, 174-187.

Fox L, Marrow P (1983) Estimates of damage by herbivorous insects on *Eucalyptus* trees. *Australian Journal of Ecology* **8**, 139–147.

Frascaria-Lacoste N, Fernandez-Manjarres J (2012) Assisted colonization of foundation species: lack of consideration of the extended phenotype concept-response to Kreyling *et al.* (2011). *Restoration Ecology* **20**, 296-298.

Freeman J, Jackson H, Steane D, McKinnon G, Dutkowski G, Potts B, Vaillancourt R (2001) Chloroplast DNA phylogeography of *Eucalyptus globulus*. *Australian Journal of Botany* **49**, 585-596.

Freeman J, O'Reilly-Wapstra J, Vaillancourt R, Wiggins N, Potts B (2008a) Quantitative trait loci for key defensive compounds affecting herbivory of eucalypts in Australia. *New Phytologist* **178**, 846-851.

Freeman J, Potts B, Downes G, Pilbeam D, Thavamanikumar S, Vaillancourt R (2013) Stability of quantitative trait loci for growth and wood properties across multiple pedigrees and environments in *Eucalyptus globulus*. *New Phytologist* **198**, 1121-1134.

Freeman J, Potts B, Shepherd M, Vaillancourt R (2006) Parental and consensus linkage maps of *Eucalyptus globulus* using AFLP and microsatellite markers. *Silvae Genetica* **55**, 202-217.

Freeman J, Potts B, Vaillancourt R (2008b) Few Mendelian genes underlie the quantitative response of a forest tree, *Eucalyptus globulus*, to a natural fungal epidemic. *Genetics* **178**, 563-571.

Freeman J, Whittock P, Potts B, Vaillancourt R (2009) QTL influencing growth and wood properties in *Eucalyptus globulus*. *Tree Genetics & Genomes* **5**, 713-722.

Fritz R, Price P (1988) Genetic variation among plants and insect community structure: willows and sawflies. *Ecology* **69**, 845-856.

Garnas J, Hurley B, Slippers B, Wingfield M (2012) Biological control of forest plantation pests in an interconnected world requires greater international focus. *International Journal of Pest Management* **58**, 211-223.

Garrison R (1998) ' New agricultural pest for southern California: Australian tortoise beetle, *Trachymela sloanei*.' (Los Angeles County Department of Agriculture: El Monte, CA).

Gauli A, Vaillancourt R, Bailey T, Steane D, Potts B (2015) Evidence for local climate adaptation in early-life traits of Tasmanian populations of *Eucalyptus pauciflora*. *Tree Genetics & Genomes* **11**, 1-14.

Gehring C, Flores-Rentería D, Sthultz C, Leonard T, Flores-Rentería L, Whipple A, Whitham T (2014) Plant genetics and interspecific competitive interactions determine ectomycorrhizal fungal community responses to climate change. *Molecular Ecology* **23**, 1379-1391.

Gershenzon J, Dudareva N (2007) The function of terpene natural products in the natural world. *Nature Chemical Biology* **3**, 408-414.

Gilmour A, Thompson R, Cullis B, Welham S (2009) 'ASReml user guide release 3.0.' (VSN International Ltd: Hemel Hempstead, UK).

Glen M, Smith A, Langrell S, Mohammed C (2007) Development of nested polymerase chain reaction detection of *Mycosphaerella* spp. and its application to the study of leaf disease in *Eucalyptus* plantations. *Phytopathology* **97**, 132-144.

Gomes De Oliveira H, Molina-Rugama A, M. Fadini M, Rezende D, Soto G A, Oliveira C, Pallini A (2010) Induced defense in *Eucalyptus* trees increases with prolonged herbivory. *Revista Colombiana de Entomología* **36**, 1-4.

Gosney B, O'Reilly-Wapstra J, Forster L, Barbour R, Iason G, Potts B (2014) Genetic and ontogenetic variation in an endangered tree structures dependent arthropod and fungal communities. *PloS One* **9**, e114132.

Gosney B, Potts B, O'Reilly-Wapstra J, Vaillancourt R, Fitzgerald H, Davies N, Freeman J (2016) Genetic control of cuticular wax compounds in *Eucalyptus globulus*. *New Phytologist* **209**, 202-215.

Grattapaglia D, Plomion C, Kirst M, Sederoff R (2009) Genomics of growth traits in forest trees. *Current opinion in plant biology* **12**, 148-156.

Gray L, Hamann A, John S, Rweyongeza D, Barnhardt L, Thomas B (2016) Climate change risk management in tree improvement programs: selection and movement of genotypes. *Tree Genetics & Genomes* **12**, 1-12.

Green J (1969) Temperature responses in altitudinal populations of *Eucalyptus pauciflora* Sieb. ex Spreng. *New Phytologist* **68**, 399-410.

Hall D, Matus A, Lamberton JA, Barber H (1965) Infra-specific variation in wax on leaf surfaces. *Australian Journal of Biological Sciences* **18**, 323-332.

Hamanishi E, Campbell M (2011) Genome-wide responses to drought in forest trees. *Forestry* **84**, 273-283.

Hamilton M, Williams D, Tilyard P, Pinkard E, Wardlaw T, Glen M, Vaillancourt R, Potts B (2013) A latitudinal cline in disease resistance of a host tree. *Heredity* **110**, 372-379.

Hamrick J (1976) Variation and selection in western montane species II. Variation within and between populations of white firon an elevational transect. *Theoretical and Applied Genetics* **47**, 27-34.

Hamrick J (1989) Isozymes and the analysis of genetic structure in plant populations. In 'Isozymes in Plant Biology'. pp. 87-105. (Springer).

Harrison P, Vaillancourt R, Harris R, Potts B (2017) Integrating climate change and habitat fragmentation to identify candidate seed sources for ecological restoration. *Restoration Ecology*.

Heimonen K, Valtonen A, Kontunen-Soppela S, Keski-Saari S, Rousi M, Oksanen E, Roininen H (2017) Susceptibility of silver birch (*Betula pendula*) to herbivorous insects is associated with the size and phenology of birch–implications for climate warming. *Scandinavian Journal of Forest Research* **32**, 1-10.

Henery M, Henson M, Wallis I, Stone C, Foley W (2008) Predicting crown damage to *Eucalyptus grandis* by *Paropsis atomaria* with direct and indirect measures of leaf composition. *Forest Ecology and Management* **255**, 3642-3651.

Henery M, Moran G, Wallis I, Foley W (2007) Identification of quantitative trait loci influencing foliar concentrations of terpenes and formylated phloroglucinol compounds in *Eucalyptus nitens*. *New Phytologist* **176**, 82-95.

Hersch-Green E, Turley N, Johnson M (2011) Community genetics: what have we accomplished and where should we be going? *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **366**, 1453-1460.

Hillis W (1984) 'Eucalypts for wood production.' (Commonwealth Scientific and Industrial Research Organization-Academic Press Australia).

Hobbs R (2007) Setting effective and realistic restoration goals: key directions for research. *Restoration Ecology* **15**, 354-357.

Hochwender C, Fritz R (2004) Plant genetic differences influence herbivore community structure: evidence from a hybrid willow system. *Oecologia* **138**, 547-557.

Hocking R (1976) The analysis and Selection of variables in linear regression. *Biometrics* **32**, 1-49.

Hoffmann B, Kahmen A, Cernusak L, Arndt S, Sachse D (2013) Abundance and distribution of leaf wax n-alkanes in leaves of *Acacia* and *Eucalyptus* trees along a strong humidity gradient in northern Australia. *Organic Geochemistry* **62**, 62-67.

Holeski L, Hillstrom M, Whitham T, Lindroth R (2012) Relative importance of genetic, ontogenetic, induction, and seasonal variation in producing a multivariate defense phenotype in a foundation tree species. *Oecologia* **170**, 695-707.

Hudson C, Freeman J, Jones R, Potts B, Wong M, Weller J, Hecht V, Poethig R, Vaillancourt R (2014) Genetic control of heterochrony in *Eucalyptus globulus*. *G3: Genes, Genomes, Genetics* **4**, 1235-1245.

Hudson C, Freeman J, Kullan A, Petroli C, Sansaloni C, Kilian A, Detering F, Grattapaglia D, Potts B, Myburg A, Vaillancourt R (2012a) A reference linkage map for *Eucalyptus*. *BMC Genomics* **13**, 240.

Hudson C, Kullan A, Freeman J, Faria D, Grattapaglia D, Kilian A, Myburg A, Potts B, Vaillancourt R (2012b) High synteny and colinearity among Eucalyptus genomes revealed by high-density comparative genetic mapping. *Tree Genetics & Genomes* **8**, 339–352.

Islam M, Du H, Ning J, Ye H, Xiong L (2009) Characterization of Glossy1-homologous genes in rice involved in leaf wax accumulation and drought resistance. *Plant molecular biology* **70**, 443-456.

Jairus T, Mpumba R, Chinoya S, Tedersoo L (2011) Invasion potential and host shifts of Australian and African ectomycorrhizal fungi in mixed eucalypt plantations. *New Phytologist* **192**, 179-187.

James S, Bell D (2001) Leaf morphological and anatomical characteristics of heteroblastic *Eucalyptus globulus* ssp. *globulus* (Myrtaceae). *Australian Journal of Botany* **49**, 259-269.

Jarrold S, Moore D, Potter U, Charnley A (2007) The contribution of surface waxes to pre-penetration growth of an entomopathogenic fungus on host cuticle. *Mycological Research* **111**, 240-249.

Jeffree C, Sandford A (1982) Crystalline structure of plant epicuticular waxes demonstrated by cryostage scanning electron microscopy. *New Phytologist* **91**, 549-559.

Jenks M, Rashotte A, Tuttle H, Feldmann K (1996) Mutants in *Arabidopsis thaliana* altered in epicuticular wax and leaf morphology. *Plant Physiology* **110**, 377-385.

Johnson M (2008) Bottom-up effects of plant genotype on aphids, ants, and predators. *Ecology* **89**, 145-154.

Johnson M, Argawal A (2007) Covariation and composition of arthropod species across plant genotypes of evening primrose, *Oenothera biennis*. *Oikos* **116**, 941-956.

Johnson M, Lajeunesse M, Agrawal A (2006) Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. *Ecology letters* **9**, 24-34.

Jones R, McKinnon G, Potts B, Vaillancourt R (2005) Genetic diversity and mating system of an endangered tree *Eucalyptus morrisbyi*. *Australian Journal of Botany* **53**, 367-377.

Jones R, Steane D, Lavery M, Vaillancourt R, Potts B (2013) Multiple evolutionary processes drive the patterns of genetic differentiation in a forest tree species complex. *Ecology and Evolution* **3**, 1-17.

Jones R, Vaillancourt R, Gore P, Potts B (2011) Genetic control of flowering time in *Eucalyptus globulus* ssp *globulus*. *Tree Genetics & Genomes* **7**, 1209-1218.

Jones R, Wiltshire R, Wright M, Visby I, Aubrey M, Vaillancourt R, Potts B (2016) *Eucalyptus morrisbyi*: one of Australia's most endangered eucalypts. Poster presented at Bjarne K Dahl Trust Symposium "Conserving eucalypts: the why and the how", Royal Society of Victoria, Melbourne, 18 March.

Jones T (2013) When local isn't best. *Evolutionary Applications* **6**, 1109-1118.

Jones T, Potts B, Vaillancourt R, Davies N (2002) Genetic resistance of *Eucalyptus globulus* to autumn gum moth defoliation and the role of cuticular waxes. *Canadian Journal of Forest Research* **32**, 1961-1969.

Jordan G, Potts B, Clarke A (2002) Susceptibility of *Eucalyptus globulus* ssp *globulus* to sawfly (*Perga affinis ssp insularis*) attack and its potential impact on plantation productivity. *Forest Ecology and Management* **160**, 189-199.

Kannangara R, Branigan C, Liu Y, Penfield T, Rao V, Mouille G, Höfte H, Pauly M, Riechmann J, Broun P (2007) The transcription factor *WIN1/SHN1* regulates cutin biosynthesis in *Arabidopsis thaliana*. *The Plant Cell* **19**, 1278-1294.

Karageorgou P, Manetas Y (2006) The importance of being red when young: anthocyanins and the protection of young leaves of *Quercus coccifera* from insect herbivory and excess light. *Tree Physiology* **26**, 613-621.

Katerinopoulos H, Pagona G, Afratis A, Stratigakis N, Roditakis N (2005) Composition and insect attracting activity of the essential oil of *Rosmarinus officinalis*. *Journal of Chemical Ecology* **31**, 111-122.

Keith A, Bailey J, Whitham T (2010) A genetic basis to community repeatability and stability. *Ecology* **91**, 3398-3406.

Kliebenstein D, Pedersen D, Barker B, Mitchell-Olds T (2002) Comparative analysis of quantitative trait loci controlling glucosinolates, myrosinase and insect resistance in *Arabidopsis thaliana*. *Genetics* **161**, 325-332.

Kolattukudy P (1996) Biosynthetic pathways of cutin and waxes, and their sensitivity to environmental stresses. In 'Plant Cuticles'. (Ed. G Kerstiens) pp. 83-108. (BIOS Scientific publishers Ltd.: Oxford).

Korkama-Rajala T, Müller M, Pennanen T (2007) Decomposition and fungi of needle litter from slow- and fast-growing Norway spruce (*Picea abies*) clones. *Microbial Ecology* **56**, 76-89.

Korkama T, Pakkanen A, Pennanen T (2006) Ectomycorrhizal community structure varies among Norway spruce (*Picea abies*) clones. *New Phytologist* **171**, 815-824.

Kraus T, Dahlgren R, Zasoski R (2003) Tannins in nutrient dynamics of forest ecosystems - a review. *Plant and Soil* **256**, 41-66.

Kremer A, Potts B, Delzon S (2014) Genetic divergence in forest trees: understanding the consequences of climate change. *Functional Ecology* **28**, 22-36.

Kunst L, Samuels A (2003) Biosynthesis and secretion of plant cuticular wax. *Progress in Lipid Research* **42**, 51-80.

Kursar T, Coley P (1992) Delayed greening in tropical leaves: an antiherbivore defense? *Biotropica*, 256-262.

Lam P, Zhao L, McFarlane H, Aiga M, Lam V, Hooker T, Kunst L (2012) RDR1 and SGS3, components of RNA-mediated gene silencing, are required for the regulation of cuticular wax biosynthesis in developing inflorescence stems of *Arabidopsis*. *Plant Physiology* **159**, 1385-1395.

Lamers Y, Rusu G, Wijnberg J, de Groot A (2003) Synthesis of chiral methyl-branched linear pheromones starting from (+)-aromadendrene. Part 7. *Tetrahedron* **59**, 9361-9369.

Lamit L, Busby P, Lau M, Compson Z, Wojtowicz T, Keith A, Zinkgraf M, Schweitzer J, Shuster S, Gehring C (2015) Tree genotype mediates covariance among communities from microbes to lichens and arthropods. *Journal of Ecology* **103**, 840-850.

Lander E, Botstein D (1989) Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* **121**, 185-199.

Larson K, Whitham T (1997) Competition between gall aphids and natural plant sinks: plant architecture affects resistance to galling. *Oecologia* **109**, 575-582.

Lau M, Keith A, Borrett S, Shuster S, Whitham T (2016) Genotypic variation in foundation species generates network structure that may drive community dynamics and evolution. *Ecology* **97**, 733-742.

Lawrence R, Potts B, Whitham T (2003) Relative importance of plant ontogeny, host genetic variation, and leaf age for a common herbivore. *Ecology* **84**, 1171-1178.

Le Provost G, Domergue F, Lalanne C, Campos P, Grosbois A, Bert D, Meredieu C, Danjon F, Plomion C, Gion J (2013) Soil water stress affects both cuticular wax content and cuticle-related gene expression in young saplings of maritime pine (*Pinus pinaster* Ait). *BMC plant biology* **13**, 95.

Lee S, Suh M (2013) Recent advances in cuticular wax biosynthesis and its regulation in *Arabidopsis*. *Molecular Plant* **6**, 246-249.

Li-Beisson Y, Shorrosh B, Beisson F, Andersson M, Arondel V, Bates P, Baud S, Bird D, DeBono A, Durrett T, Franke R, Graham I, Katayama K, Kelly A, Larson T, Markham J, Miquel M, Molina I, Nishida I, Rowland O, Samuels L, Schmid K, Wada H, Welti R, Xu C, Zallot R, Ohlrogge J (2010) Acyl-Lipid Metabolism. *The Arabidopsis Book / American Society of Plant Biologists* **8**, e0133.

Li H, Madden J, Potts B (1996) Variation in volatile leaf oils of the Tasmanian *Eucalyptus* species II. subgenus *Symphyomyrtus*. *Biochemical Systematics and Ecology* **24**, 547-569.

Li H, Madden J, Potts B (1997) Variation in leaf waxes of the Tasmanian *Eucalyptus* species I. Subgenus *Symphyomyrtus*. *Biochemical Systematics and Ecology* **25**, 631-657.

Loch A, Floyd R (2001) Insect pests of Tasmanian blue gum, *Eucalyptus globulus globulus*, in south-western Australia: History, current perspectives and future prospects. *Austral Ecology* **26**, 458-466.

Loch A, Matsuki M (2010) Effects of defoliation by *Eucalyptus* weevil, *Gonipterus scutellatus*, and chrysomelid beetles on growth of *Eucalyptus globulus* in southwestern Australia. *Forest Ecology and Management* **260**, 1324-1332.

Lokesh U, Kiranmai K, Pandurangaiah M, Sudhakarbabu O, Nareshkumar A, Sudhakar C (2013) Role of plant fatty acid elongase (3 keto acyl-CoA Synthase) gene in cuticular wax biosynthesis. *Journal of Agriculture and Allied Sciences* **2**, 35-42.

Macauley B, Fox L (1980) Variation in total phenols and condensed tannins in *Eucalyptus*: leaf phenology and insect grazing. *Australian Journal of Ecology* **5**, 31-35.

Macias F, Galindo J, Galindo J (2007) Evolution and current status of ecological phytochemistry. *Phytochemistry* **68**, 2917-2936.

Mackay T, Stone E, Ayroles J (2009) The genetics of quantitative traits: challenges and prospects. *Nature Reviews Genetics* **10**, 565-577.

Maddox G, Root R (1987) Resistance to 16 diverse species of herbivorous insects within a population of goldenrod, *Solidago altissima*: genetic variation and heritability. *Oecologia* **72**, 8-14.

Maherali H, Caruso C, Sherrard M (2009) The adaptive significance of ontogenetic changes in physiology: a test in *Avena barbata*. *New Phytologist* **183**, 908-918.

Maldonado-Lopez Y, Cuevas-Reyes P, González-Rodríguez A, Pérez-López G, Acosta-Gómez C, Oyama K (2015) Relationships among plant genetics, phytochemistry and

herbivory patterns in *Quercus castanea* across a fragmented landscape. *Ecological Research* **30**, 133-143.

Mann A, O'Reilly-Wapstra J, Iason G, Sanson G, Davies N, Tilyard P, Williams D, Potts B (2012) Mammalian herbivores reveal marked genetic divergence among populations of an endangered plant species. *Oikos* **121**, 268-276.

Marques W, Salazar M, Camargo E, Lepikson-Neto J, Tiburcio R, do Nascimento L, Pereira G (2012) Identification of four *Eucalyptus* genes potentially involved in cell wall biosynthesis and evolutionarily related to *SHINE* transcription factors. *Plant Growth Regulation* **69**, 203-208.

Matsuki M, Foley W, Floyd R (2011) Role of volatile and non-volatile plant secondary metabolites in host tree selection by christmas beetles. *Journal of Chemical Ecology* **37**, 286-300.

Mauricio R (2001) Mapping quantitative trait loci in plants: uses and caveats for evolutionary biology. *Nature Reviews Genetics* **2**, 370-381.

Mayr E (1982) 'The growth of biological thought: Diversity, evolution, and inheritance.' (Harvard University Press).

Milgate A, Potts B, Joyce K, Mohammed C, Vaillancourt R (2005a) Genetic variation in *Eucalyptus globulus* for susceptibility to *Mycosphaerella nubilosa* and its association with tree growth. *Australasian Plant Pathology* **34**, 11-18.

Milgate A, Vaillancourt R, Mohammed C, Powell M, Potts B (2005b) Genetic structure of a *Mycosphaerella cryptica* population. *Australasian Plant Pathology* **34**, 345-354.

Mintz-Oron S, Mandel T, Rogachev I, Feldberg L, Lotan O, Yativ M, Wang Z, Jetter R, Venger I, Adato A (2008) Gene expression and metabolism in tomato fruit surface tissues. *Plant Physiology* **147**, 823-851.

Mithöfer A, Maffei M (2016) General mechanisms of plant defense and plant toxins. In 'Plant Toxins'. pp. 1-22. (Springer).

Møller A, Jennions M (2002) How much variance can be explained by ecologists and evolutionary biologists? *Oecologia* **132**, 492-500.

Mooney K, Agrawal A (2008) Plant genotype shapes ant-aphid interactions: implications for community structure and indirect plant defense. *The American Naturalist* **171**, E195-E205.

Moore B, Wallis I, Palá-Paúl J, Brophy J, Willis R, Foley W (2004) Antiherbivore chemistry of *Eucalyptus*--cues and deterrents for marsupial folivores. *Journal of chemical ecology* **30**, 1743-1769.

Morgenstern M (2011) 'Geographic variation in forest trees: genetic basis and application of knowledge in silviculture.' (UBC press: Vancouver, Canada).

Mousseau T, Sinervo B, Endler J (2000) 'Adaptive genetic variation in the wild.' (Oxford University Press on Demand).

Müller F (1996) Emergent properties of ecosystems-consequences of self-organizing processes? *Senckenbergiana maritima* **27**, 151-168.

Mulyaningsih S, Sporer F, Zimmermann S, Reichling J, Wink M (2010) Synergistic properties of the terpenoids aromadendrene and 1,8-cineole from the essential oil of *Eucalyptus globulus* against antibiotic-susceptible and antibiotic-resistant pathogens. *Phytomedicine* **17**, 1061-1066.

Munck L (2007) A new holistic exploratory approach to systems biology by near infrared spectroscopy evaluated by chemometrics and data inspection. *Journal of Chemometrics* **21**, 406-426.

Murray B, Baker A, Robson T (2010) Impacts of the replacement of native woodland with exotic pine plantations on leaf-litter invertebrate assemblages: a test of a novel framework. *International Journal of Ecology* **2009**, 490395.

Myburg A, Grattapaglia D, Tuskan G, Hellsten U, Hayes R, Grimwood J, Jenkins J, Lindquist E, Tice H, Bauer D (2014) The genome of Eucalyptus grandis. *Nature* **510**, 356-362.

Neinhuis C, Barthlott W (1997) Characterization and distribution of water-repellent, self-cleaning plant surfaces. *Annals of Botany* **79**, 667–677.

Nicolle D (2006) 'Eucalypts of Victoria and Tasmania.' (Bloomings Books).

Nielsen S, Müller F (2000) 11.2. 2 Emergent properties of ecosystems. In 'Handbook of Ecosystem Theories and Management'. pp. 195. (CRC Press).

Norris C, Preston C, Hogg K, Titus B (2011) The influence of condensed tannin structure on rate of microbial mineralization and reactivity to chemical assays. *Journal of Chemical Ecology* **37**, 311-319.

Novotny V, Basset Y, Miller S, Weiblen G, Bremer B, Cizek L, Drozd P (2002) Low host specificity of herbivorous insects in a tropical forest. *Nature* **416**, 841-844.

O'Brien E, Mazanec R, Krauss S (2007) Provenance variation of ecologically important traits of forest trees: implications for restoration. *Journal of Applied Ecology* **44**, 583-593.

O'Reilly-Wapstra J, Freeman J, Barbour R, Vaillancourt R, Potts B (2013a) Genetic analysis of the near-infrared spectral phenome of a global *Eucalyptus* species. *Tree Genetics & Genomes* **9**, 943-959.

O'Reilly-Wapstra J, Freeman J, Davies N, Vaillancourt R, Fitzgerald H, Potts B (2011) Quantitative trait loci for foliar terpenes in a global eucalypt species. *Tree Genetics & Genomes* **7**, 485-498.

O'Reilly-Wapstra J, Humphreys J, Potts B (2007) Stability of genetic-based defensive chemistry across life stages in a *Eucalyptus* species. *Journal of Chemical Ecology* **33**, 1876-1884.

O'Reilly-Wapstra J, McArthur C, Potts B (2002) Genetic variation in resistance of *Eucalyptus globulus* to marsupial browsers. *Oecologia* **130**, 289-296.

O'Reilly-Wapstra J, McArthur C, Potts B (2004) Linking plant genotype, plant defensive chemistry and mammal browsing in a *Eucalyptus* species. *Functional Ecology* **18**, 677-684.

O'Reilly-Wapstra J, Miller A, Hamilton M, Williams D, Glancy-Dean N, Potts B (2013b) Chemical variation in a dominant tree species: population divergence, selection and genetic stability across environments. *PloS One* **8**, e58416.

O'Reilly-Wapstra J, Bailey J, McArthur C, Potts B (2010) Genetic-and chemical-based resistance to two mammalian herbivores varies across the geographic range of *Eucalyptus globulus*. *Evolutionary Ecology Research* **12**, 491-505.

Oyedeji A, Ekundayo O, Olawore O, Adeniyi B, Koenig W (1999) Antimicrobial activity of the essential oils of five *Eucalyptus* species growing in Nigeria. *Fitoterapia* **70**, 526-528.

Padovan A, Keszei A, Foley W, Külheim C (2013) Differences in gene expression within a striking phenotypic mosaic *Eucalyptus* tree that varies in susceptibility to herbivory. *BMC plant biology* **13**, 1.

Padovan A, Keszei A, Köllner T, Degenhardt J, Foley W (2010) The molecular basis of host plant selection in *Melaleuca quinquenervia* by a successful biological control agent. *Phytochemistry* **71**, 1237-1244.

Paine T, Steinbauer M, Lawson S (2011) Native and exotic pests of *Eucalyptus*: a worldwide perspective. *Annual Review of Entomology* **56**, 181-201.

Park R, Keane P (1982) Leaf diseases of Eucalyptus associated with Mycosphaerella species. *Transactions of the British Mycological Society* **79**, 101-115.

Parsons R, Cameron D (1974) Maximum plant species diversity in terrestrial communities. *Biotropica (The Association for Tropical Biology and Conservation)* **6**, 202.

Pérez-López G, González-Rodríguez A, Oyama K, Cuevas-Reyes P (2016) Effects of plant hybridization on the structure and composition of a highly rich community of cynipid gall wasps: the case of the oak hybrid complex *Quercus magnoliifolia* x *Quercus resinosa* in Mexico. *Biodiversity and Conservation* **25**, 633-651.

Pinkard E, Mohammed C (2006) Photosynthesis of *Eucalyptus globulus* with *Mycosphaerella* leaf disease. *New Phytologist* **170**, 119-127.

Plummer M (2003) JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling. Proceedings of the 3rd International Workshop on Distributed Statistical Computing, p. 125

Post-Beittenmiller D (1996) Biochemistry and molecular biology of wax production in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **47**, 405-430.

Potts B, Jackson W (1986) Evolutionary processes in the Tasmanian high altitude eucalypts. In 'Flora and Fauna of Alpine Australasia. Ages and Origins'. (Ed. B Barlow) pp. 511-527. (CSIRO: Melbourne, Australia).

Potts B, Sandhu K, Wardlaw T, Freeman J, Li H, Tilyard P, Park R (2016) Evolutionary history shapes the susceptibility of an island tree flora to an exotic pathogen. *Forest Ecology and Management* **368**, 183-193.

Potts B, Vaillancourt R, Jordan G, Dutkowski G, McKinnon G, Steane D, Volker P, Lopez G, Apiolaza L, Li Y (2004) Exploration of the *Eucalyptus globulus* gene pool. In '*Eucalyptus* in a Changing World. ' (Eds N Borrahlo, J Pereira, C Marques, J Coutinho, M Madeira and M Tomé) pp. 46-61. (RAIZ, Instituto Investigação de Floresta e Papel: Aceiro, Portugal)

Price A (2006) Believe it or not, QTLs are accurate! *Trends in Plant Science* **11**, 213-216.

Prober S, Potts B, Bailey T, Byrne M, Dillon S, Harrison P, Hoffmann A, Jordan R, McLean E, Steane D (2016) Climate adaptation and ecological restoration in eucalypts. *Proceedings of the Royal Society of Victoria* **128**, 40-53.

Pryor L (1956) Variation in snow gum (*Eucalyptus pauciflora* Sieb.). Proceedings of the Linnean Society of New South Wales, pp. 299-305

Quinn G, Keough M (2002) 'Experimental design and data analysis for biologists.' (Cambridge University Press: Cambridge, UK).

R Core Team (2013) 'R: A language and environment for statistical computing.' (R Foundation for Statistical Computing: Vienna, Austria).

Rae A, Street N, Robinson K, Harris N, Taylor G (2009) Five QTL hotspots for yield in short rotation coppice bioenergy poplar: The Poplar biomass loci. *BMC Plant Biology* **9**, 1-13.

Ramirez-Herrera C, Percy K, Yeates L, Vargas-Hernandez J (2010) Genetic variation in leaf epicuticular wax characteristics in *Pinus pinceana* seedlings. *Silvae Genetica* **60**, 210-215.

Rapley L, Allen G, Potts B (2004a) Genetic variation in *Eucalyptus globulus* in relation to susceptibility from attack by the southern eucalypt leaf beetle, *Chrysophtharta agricola*. *Australian Journal of Botany* **52**, 747-756.

Rapley L, Allen G, Potts B (2004b) Susceptibility of *Eucalyptus globulus* to *Mnesampela privata* defoliation in relation to a specific foliar wax compound. *Chemoecology* **14**, 157-163.

Rapley L, Allen G, Potts B, Davies N (2007) Constitutive or induced defences - how does *Eucalyptus globulus* defend itself from larval feeding? *Chemoecology* **17**, 235-243.

Rashotte A, Jenks M, Feldmann K (1997) Epicuticular wax variation in ecotypes of *Arabidopsis thaliana*. *Phytochemistry* **45**, 251-255.

Ratnam W, Rajora O, Finkeldey R, Aravanopoulos F, Bouvet J, Vaillancourt R, Kanashiro M, Fady B, Tomita M, Vinson C (2014) Genetic effects of forest management practices: global synthesis and perspectives. *Forest Ecology and Management* **333**, 52-65.

Raven J, Edwards D (2004) Physiological evolution of lower embryophytes: adaptations to the terrestrial environment. In 'The Evolution of Plant Physiology'. Vol. 21. (Eds A Hemsley and I Poole) pp. 17-41. (Elsevier Academic Press: Amsterdam).

Raymond C (1995) Genetic variation in *Eucalyptus regnans* and *Eucalyptus nitens* for levels of observed defoliation caused by the eucalyptus leaf-beetle, *Chrysophtharta bimaculata olivier*, in Tasmania. *Forest Ecology and Management* **72**, 21-29.

Reisige K, Gorzelanny C, Daniels U, Moerschbacher B (2006) The C28 aldehyde octacosanal is a morphogenetically active component involved in host plant recognition and infection structure differentiation in the wheat stem rust fungus. *Physiological and Molecular Plant Pathology* **68**, 33-40.

Reuter H, Hölker F, Middelhoff U, Jopp F, Eschenbach C, Breckling B (2005) The concepts of emergent and collective properties in individual-based models—Summary and outlook of the Bornhöved case studies. *Ecological Modelling* **186**, 489-501.

Riederer M, Müller C (2006) 'Biology of the Plant Cuticle.' (Wiley-Blackwell: Germany).

Riederer M, Schreiber L (1995) Waxes: the transport barriers of plant cuticles. In 'Waxes: Chemistry, Molecular Biology and Functions'. Vol. 6. (Ed. R Hamilton) pp. 130-156. (The Oily Press: Dundee, Scotland).

Riederer M, Schreiber L (2001) Protecting against water loss: analysis of the barrier properties of plant cuticles. *Journal of Experimental Botany* **52**, 2023-2032.

Robinson K, Ingvarsson P, Jansson S, Albrectsen B (2012) Genetic variation in functional traits influences arthropod community composition in aspen (*Populus tremula* L.). *PloS One* **7**, e37679.

Roche B, Fritz R (1997) Genetics of resistance of *Salix sericea* to a diverse community of herbivores. *Evolution* **51**, 1490-1498.

Rönnberg-Wästljung A, Glynn C, Weih M (2005) QTL analyses of drought tolerance and growth for a *Salix dasyclados* × *Salix viminalis* hybrid in contrasting water regimes. *Theoretical and Applied Genetics* **110**, 537-549.

Rönnberg-Wästljung A, Åhman I, Glynn C, Widenfalk O (2006) Quantitative trait loci for resistance to herbivores in willow: field experiments with varying soils and climates. *Entomologia Experimentalis et Applicata* **118**, 163-174.

Rosenthal G, Berenbaum M (2012) 'Herbivores: Their interactions with secondary plant metabolites.' (Academic Press Inc: San Diego, California).

Salazar D, Marquis R (2012) Herbivore pressure increases toward the equator. *Proceedings of the National Academy of Sciences* **109**, 12616-12620.

Samuels L, Kunst L, Jetter R (2008) Sealing plant surfaces: cuticular wax formation by epidermal cells. *Annual Review of Plant Biology* **59**, 683-707.

Schimel J, Cleve K, Cates R, Clausen T, Reichardt P (1996) Effects of balsam poplar (*Populus balsamifera*) tannins and low molecular weight phenolics on microbial activity in taiga floodplain soil: implications for changes in N cycling during succession. *Canadian Journal of Botany* **74**, 84-90.

Schweitzer J, Bailey J, Fischer D, LeRoy C, Lonsdorf E, Whitham T, Hart S (2008a) Plant–soil–microorganism interactions: heritable relationship between plant genotype and associated soil microorganisms. *Ecology* **89**, 773-781.

Schweitzer J, Madritch M, Bailey J, LeRoy C, Fischer D, Rehill B, Lindroth R, Hagerman A, Wooley S, Hart S (2008b) From genes to ecosystems: the genetic basis of condensed tannins and their role in nutrient regulation in a *Populus* model system. *Ecosystems* **11**, 1005-1020.

Sela D, Buxdorf K, Shi J, Feldmesser E, Schreiber L, Aharoni A, Levy M (2013) Overexpression of *AtSHN1/WIN1* provokes unique defense responses. *PloS One* **8**, e70146.

Seo P, Park C (2011) Cuticular wax biosynthesis as a way of inducing drought resistance. *Plant Signaling & Behavior* **6**, 1043-1045.

Serrano M, Coluccia F, Torres M, L'Haridon F, Métraux J (2014) The cuticle and plant defense to pathogens. *Frontiers in Plant Science* **5**, 274.

Shi J, Adato A, Alkan N, He Y, Lashbrooke J, Matas A, Meir S, Malitsky S, Isaacson T, Prusky D, Leshkowitz D, Schreiber L, Granell A, Widemann E, Grausem B, Pinot F, Rose J, Rogachev I, Rothan C, Aharoni A (2013) The tomato *SlSHINE3* transcription factor regulates fruit cuticle formation and epidermal patterning. *New Phytologist* **197**, 468-480.

Silfver T, Rousi M, Oksanen E, Roininen H (2014) Genetic and environmental determinants of insect herbivore community structure in a *Betula pendula* population. *F1000Research* **3**.

Silva Fernandes A (1965) Studies on plant cuticle VIII. Surface waxes in relation to water-repellency. *Annals of Applied Biology* **56**, 297-304.

Sinclair F, Stone G, Nicholls J, Cavers S, Gibbs M, Butterill P, Wagner S, Ducousso A, Gerber S, Petit R (2015) Impacts of local adaptation of forest trees on associations with herbivorous insects: implications for adaptive forest management. *Evolutionary Applications* **8**, 972-987.

Sivapalan K (1981) Phenolics and the exchange capacity of humic materials. *Soil Biology and Biochemistry* **13**, 331-333.

Slatkin M (2008) Linkage disequilibrium—understanding the evolutionary past and mapping the medical future. *Nature Reviews Genetics* **9**, 477-485.

Slayter R, Morrow P (1977) Altitudinal variation in the photosynthetic characteristics of snow gum, *Eucalyptus pauciflora* Sieb. ex Spreng. I. Seasonal changes under field conditions in the Snowy Mountains area of south-eastern Australia. *Australian Journal of Botany* **25**, 1-20.

Smith A (1986) Ecology of a leaf color polymorphism in a tropical forest species: habitat segregation and herbivory. *Oecologia* **69**, 283-287.

Smith A, Pinkard E, Stone C, Battaglia M, Mohammed C (2005) Precision and accuracy of pest and pathogen damage assessment in young eucalypt plantations. *Environmental monitoring and assessment* **111**, 243-256.

Smith D, Bailey J, Shuster S, Whitham T (2011) A geographic mosaic of trophic interactions and selection: trees, aphids and birds. *Journal of Evolutionary Biology* **24**, 422-429.

Spiegelhalter D, Best N, Carlin B, Van Der Linde A (2002) Bayesian measures of model complexity and fit. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)* **64**, 583-639.

Stackpole D, Vaillancourt R, Alves A, Rodrigues J, Potts B (2011) Genetic variation in the chemical components of *Eucalyptus globulus* wood. *G3-Genes Genomes Genetics* **1**, 151-159.

Steane D, Conod N, Jones R, Vaillancourt R, Potts B (2006) A comparative analysis of population structure of a forest tree, *Eucalyptus globulus* (Myrtaceae), using microsatellite markers and quantitative traits. *Tree Genetics & Genomes* **2**, 30-38.

Steinbauer M, Clarke A, Paterson S (1998) Changes in eucalypt architecture and the foraging behaviour and development of *Amorbus obscuricornis* (*Hemiptera*: *Coreidae*). *Bulletin of Entomological Research* **88**, 641-651.

Steinbauer M, Schiestl F, Davies N (2004) Monoterpenes and epicuticular waxes help female autumn gum moth differentiate between waxy and glossy *Eucalyptus* and leaves of different ages. *Journal of Chemical Ecology* **30**, 1117-1142.

Stone C, Bacon P (1994) Relationships among moisture stress, insect herbivory, foliar cineole content and the growth of river red gum *Eucalyptus camaldulensis*. *Journal of Applied Ecology* **31**, 604-612.

Street N, Skogström O, Sjödin A, Tucker J, Rodríguez-Acosta M, Nilsson P, Jansson S, Taylor G (2006) The genetics and genomics of the drought response in *Populus*. *The Plant Journal* **48**, 321-341.

Sturaro M, Hartings H, Schmelzer E, Velasco R, Salamini F, Motto M (2005) Cloning and characterization of *GLOSSY1*, a maize gene involved in cuticle membrane and wax production. *Plant Physiology* **138**, 478-489.

Tack A, Johnson M, Roslin T (2012) Sizing up community genetics: it's a matter of scale. *Oikos* **121**, 481-488.

Tack A, Ovaskainen O, Pulkkinen P, Roslin T (2010) Spatial location dominates over host plant genotype in structuring an herbivore community. *Ecology* **91**, 2660-2672.

Tack A, Roslin T (2011) The relative importance of host-plant genetic diversity in structuring the associated herbivore community. *Ecology* **92**, 1594-1604.

Takahashi T, Kokubo R, Sakaino M (2004) Antimicrobial activities of eucalyptus leaf extracts and flavonoids from *Eucalyptus maculata*. *Letters in Applied Microbiology* **39**, 60-64.

Thomas C (2011) Translocation of species, climate change, and the end of trying to recreate past ecological communities. *Trends in Ecology & Evolution* **26**, 216-221.

Thompson J (2005) 'The geographic mosaic of coevolution.' (University of Chicago Press).

Threatened Species Section (2009) 'Listing Statement for *Eucalyptus morrisbyi* (morrisbys gum).' (Department of Primary Industries and Water: Tasmania) Available at http://dpipwe.tas.gov.au/Documents/Eucalyptus-morrisbyi-listing-statement.pdf [Verified 2 Feb 2017]

Tovar-Sanchez E, Oyama K (2006) Community structure of canopy arthropods associated to *Quercus crassifolia* x *Quercus crassipes* complex. *Oikos* **112**, 370-381.

Tsarouhas V, Gullberg U, Lagercrantz U (2004) Mapping of quantitative trait loci (QTLs) affecting autumn freezing resistance and phenology in *Salix*. *Theoretical and Applied Genetics* **108**, 1335-1342.

Tucker D, Wallis I, Bolton J, Marsh K, Rosser A, Brereton I, Nicolle D, Foley W (2010) A metabolomic approach to identifying chemical mediators of mammal–plant interactions. *Journal of Chemical Ecology* **36**, 727–735.

Uppalapati S, Ishiga Y, Doraiswamy V, Bedair M, Mittal S, Chen J, Nakashima J, Tang Y, Tadege M, Ratet P (2012) Loss of abaxial leaf epicuticular wax in *Medicago truncatula* irg1/palm1 mutants results in reduced spore differentiation of anthracnose and nonhost rust pathogens. *The Plant Cell* **24**, 353-370.

Van Ooijen J (2009) 'MapQTL 6: Software for the mapping of quantitative trait loci in experimental populations of diploid species.' (Plant Research International: Wageningen, Netherlands).

Van Poecke R, Posthumus M, Dicke M (2001) Herbivore-induced volatile production by *Arabidopsis thaliana* leads to attraction of the parasitoid *Cotesia rubecula*: chemical, behavioral, and gene-expression analysis. *Journal of Chemical Ecology* **27**, 1911-1928.

Vandegehuchte M, De La Peña E, Bonte D (2011) Contrasting covariation of above-and belowground invertebrate species across plant genotypes. *Journal of Animal Ecology* **80**, 148-158.

Vandegehuchte M, De La PeÑA E, Breyne P, Bonte D (2012) Non-local genotypes of a resident grass species reduce invertebrate species richness. *Insect Conservation and Diversity* **5**, 453-460.

Velmala S, Rajala T, Haapanen M, Taylor A, Pennanen T (2013) Genetic host-tree effects on the ectomycorrhizal community and root characteristics of Norway spruce. *Mycorrhiza* **23**, 21-33.

Wallis I, Watson M, Foley W (2002) Secondary metabolites in *Eucalyptus melliodora*: field distribution and laboratory feeding choices by a generalist herbivore, the common brushtail possum. *Australian Journal of Zoology* **50**, 507-519.

Wallis IR, Keszei A, Henery ML, Moran GF, Forrester R, Maintz J, Marsh KJ, Andrew RL, Foley WJ (2011) A chemical perspective on the evolution of variation in Eucalyptus globulus. *Perspectives in Plant Ecology, Evolution and Systematics* **13**, 305-318.

Weeks A, Sgro C, Young A, Frankham R, Mitchell N, Miller K, Byrne M, Coates D, Eldridge M, Sunnucks P, Breed M, James E, Hoffmann A (2011) Assessing the benefits and risks of translocations in changing environments: a genetic perspective. *Evolutionary Applications* **4**, 709-725.

Weng H, Molina I, Shockey J (2010) Organ fusion and defective cuticle function in a lacs1lacs2 double mutant of *Arabidopsis*. *Planta* **231**, 1089-1100.

Wheeler G (2006) Chemotype variation of the weed *Melaleuca quinquenervia* influences the biomass and fecundity of the biological control agent *Oxyops vitiosa*. *Biological Control* **36**, 121-128.

Wheeler G, Ordung K (2005) Secondary metabolite variation affects the oviposition preference but has little effect on the performance of *Boreioglycaspis melaleucae*: a biological control agent of *Melaleuca quinquenervia*. *Biological Control* **35**, 115-123.

Whitham T, Bailey J, Schweitzer J, Shuster S, Bangert R, Leroy C, Lonsdorf E, Allan G, DiFazio S, Potts B, Fischer D, Gehring C, Lindroth R, Marks J, Hart S, Wimp G, Wooley S (2006) A framework for community and ecosystem genetics: from genes to ecosystems. *Nature Reviews Genetics* **7**, 510-523.

Whitham T, DiFazio S, Schweitzer J, Shuster S, Allan G, Bailey J, Woolbright S (2008) Extending genomics to natural communities and ecosystems. *Science* **320**, 492-495.

Whitham T, Gehring C, Evans L, LeRoy C, Bangert R, Schweitzer J, Allan G, Barbour R, Fischer D, Potts B, Bailey J (2010) A community and ecosystem genetics approach to conservation biology and management. In 'Molecular Approaches in Natural Resource Conservation and Management'. pp. 50-73. (Cambridge University Press: New York).

Whitham T, Gehring C, Lamit L, Wojtowicz T, Evans L, Keith A, Smith D (2012) Community specificity: life and afterlife effects of genes. *Trends in Plant Science* **17**, 271-281.

Whitlock M (2008) Evolutionary inference from QST. *Molecular Ecology* **17**, 1885-1896.

Wiggins N, McArthur C, Davies N, McLean S (2006) Behavioral responses of a generalist mammalian folivore to the physiological constraints of a chemically defended diet. *Journal of Chemical Ecology* **32**, 1133-1147.

Wiggins N, McArthur C, McLean S, Boyle R (2003) Effects of two plant secondary metabolites, cineole and gallic acid, on nightly feeding patterns of the common brushtail possum. *Journal of Chemical Ecology* **29**, 1447-1464.

Williams J, Ladiges P (1985) Morphological variation in Victorian, lowland populations of *Eucalyptus pauciflora* Sieb. ex Spreng. *Proceedings of the Royal Society of Victoria* **97**, 31-48.

Williams K, Potts B (1996) The natural distribution of *Eucalyptus* species in Tasmania. *Tasforests* **8**, 39-165.

Wiltshire R, Potts B, Reid J (1998) Genetic control of reproductive and vegetative phase change in the *Eucalyptus risdonii - E. tenuiramis* complex. *Australian Journal of Botany* **46**, 45-63.

Wimp G, Martinsen G, Floate K, Bangert R, Whitham T (2005) Plant genetic determinants of arthropod community structure and diversity. *Evolution* **59**, 61-69.

Wimp G, Wooley S, Bangert R, Young W, Martinsen G, Keim P, Rehill B, Lindroth R, Whitham T (2007) Plant genetics predicts intra-annual variation in phytochemistry and arthropod community structure. *Molecular Ecology* **16**, 5057-5069.

Wimp G, Young W, Woolbright S, Martinsen G, Keim P, Whitham T (2004) Conserving plant genetic diversity for dependent animal communities. *Ecology Letters* **7**, 776-780.

Wirthensohn M, Collins G, Jones G, Sedgley M (1999) Variability in waxiness of *Eucalyptus gunnii* foliage for floriculture. *Scientia Horticulturae* **82**, 279-288.

Wise M (2007) Evolutionary ecology of resistance to herbivory: an investigation of potential genetic constraints in the multiple-herbivore community of *Solanum carolinense*. *New Phytologist* **175**, 773-784.

Wise M (2010) Diffuse interactions between two herbivores and constraints on the evolution of resistance in horsenettle (*Solanum carolinense*). *Arthropod-Plant Interactions* **4**, 159-164.

Wolf J, Brodie III E, Cheverud J, Moore A, Wade M (1998) Evolutionary consequences of indirect genetic effects. *Trends in Ecology & Evolution* **13**, 64-69.

Wright S (2002) Plant diversity in tropical forests: a review of mechanisms of species coexistence. *Oecologia* **130**, 1-14.

Xu T (2011) 'ANUCLIM Version 6.1.' 6.1 edn. (Fenner School of Environment and Society, Australian National University: Canberra).

Yang J, Ordiz M, Jaworski J, Beachy R (2011) Induced accumulation of cuticular waxes enhances drought tolerance in *Arabidopsis* by changes in development of stomata. *Plant Physiology and Biochemistry* **49**, 1448-1455.

Yarnes C, Boecklen W, Salminen J (2008) No simple sum: seasonal variation in tannin phenotypes and leaf-miners in hybrid oaks. *Chemoecology* **18**, 39-51.

Yeats T, Rose J (2013) The formation and function of plant cuticles. *Plant Physiology* **163**, 5-20.

Yeoh S, Bell C, Foley W, Wallis I, Moran G (2012) Estimating population boundaries using regional and local-scale spatial genetic structure: an example in *Eucalyptus globulus*. *Tree Genetics & Genomes* **8**, 695–708.

Zhang Z, Wang W, Li W (2013) Genetic interactions underlying the biosynthesis and inhibition of β-diketones in wheat and their impact on glaucousness and cuticle permeability. *PloS One* **8**, e54129.

Zinkgraf M, Meneses N, Whitham T, Allan G (2016) Genetic variation in *NIN1* and *C/VIF1* genes is significantly associated with *Populus angustifolia* resistance to a galling herbivore, *Pemphigus betae*. *Journal of Insect Physiology* **84**, 50-59.

Zotz G, Wilhelm K, Becker A (2011) Heteroblasty - a review. *Botanical Review* **77**, 109-151.

Zuur A, Hilbe J, Ieno E (2013) 'A beginner's guide to GLM and GLMM with R: A frequentist and bayesian perspective for ecologists.' (Highland Statistics Newburgh).

Zytynska S, Fay M, Penney D, Preziosi R (2011) Genetic variation in a tropical tree species influences the associated epiphytic plant and invertebrate communities in a complex forest ecosystem. *Philosophical Transactions of the Royal Society B-Biological Sciences* **366**, 1329-1336.

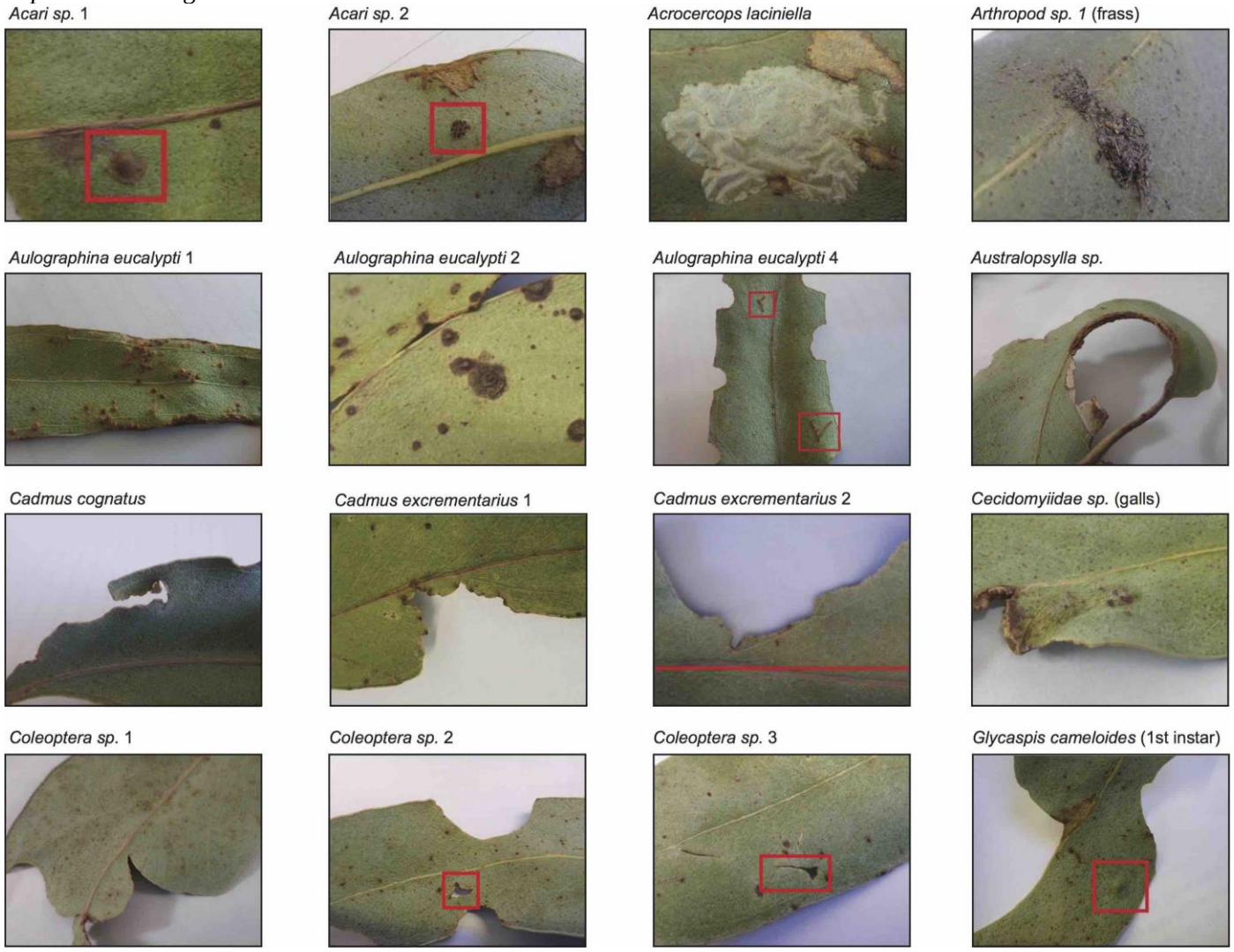
Appendix A – Supplementary material for Chapter 2

Table 2.S1. Provenances of *E. pauciflora* used in the study, including home site latitude, longitude and altitude.

Provenance	Latitude	Longitude	Altitude (m)
Bugtown Hill*	-35.52	148.44	1146
Mt. William Grampians*	-37.30	142.60	1101
Conara	-41.84	147.46	206
Lake Arthur	-41.96	146.88	1004
Great Lake	-41.99	146.70	1138
Ross	-42.00	147.53	240
Lake Leake	-42.02	147.82	597
Wihareja	-42.06	146.81	895
Interlaken	-42.15	147.14	818
Tunbridge/Woodbury†	-42.17	147.32	428
The Point	-42.19	146.42	674
Lake St. Clair	-42.20	146.14	816
Butlers Gorge	-42.28	146.33	682
Oatlands	-42.30	147.38	402
Bothwell	-42.35	146.99	468
Ellesmere/Stonor†	-42.41	147.36	433

Asterisks (*) indicate mainland Australian populations, which comprised bulk seedlots.
† indicates provenances that were combined due to low family numbers, but are considered to have an above-average nuclear genetic similarity between them (Gauli *et al*. 2014).

Fig. 2.S1. Photographs of causal organism symptoms on *Eucalyptus globulus* foliage from both Salmon River and Temma trial sites combined. Symptoms are presented in alphabetical order. Photographs for symptoms of *Acari sp.* 3, *Aulographina eucalypti* 3, *Coleoptera sp.* 4 and *Teratosphaeria sp.* 4 are missing.



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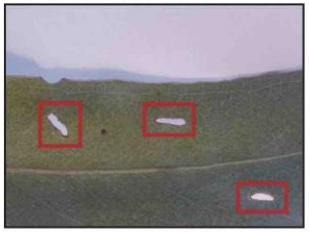
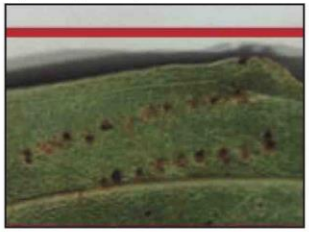
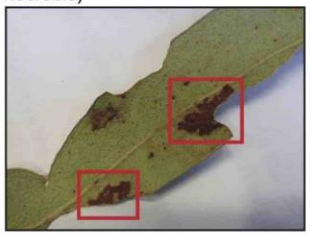
Gonipterus sp. complex



Hymenoptera sp.



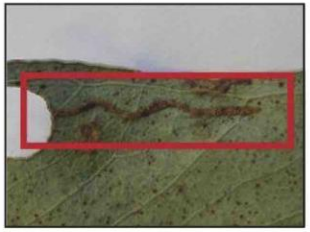
Lepidoptera grazing (followed by necrosis)



Lepidoptera: Leaf miner

Lepidoptera: Psychidae

Nepticulidae sp.



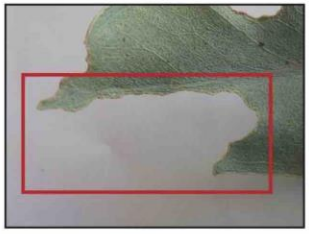
Oecophoridae sp.

Ophelimus sp. & *Myllorhinus dentifer*

Pachysacca samuelii

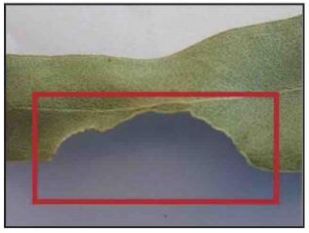
Paropsisterna agricola (egg cases and larval moult)

Paropsisterna spp.

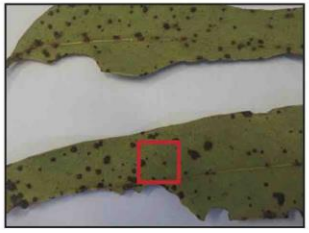


Paropsisterna sp. (larvae, early instar)

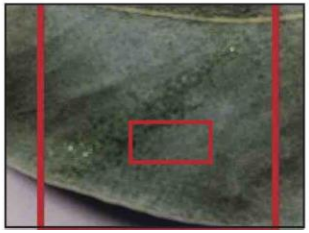
Plesanemma fucata



Pseudocercospora sp. 1



Pseudocercospora sp. 2



Pseudocercospora sp. 3

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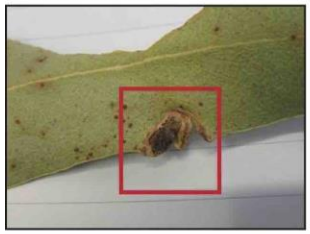
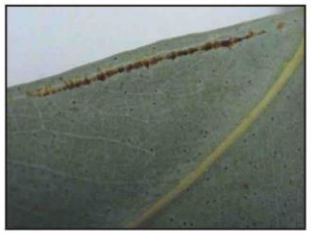
Schedotrioza sp. 1



Schedotrioza sp. 2

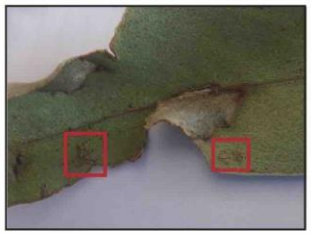


Teratosphaeria cryptica (early stage)

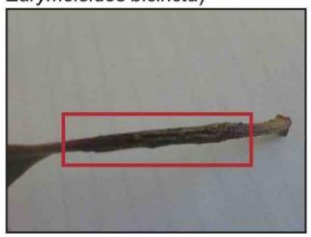
Teratosphaeria sp. 1 (like *cryptica*)

Teratosphaeria sp. 2 (following arthropod damage)

Teratosphaeria sp. 3 (and necrosis)

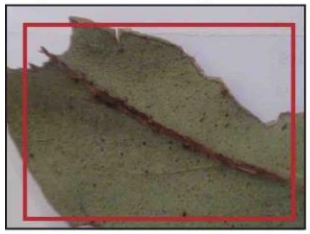


Teratosphaeria sp. 5 (following *Eurymeloides bicincta*)

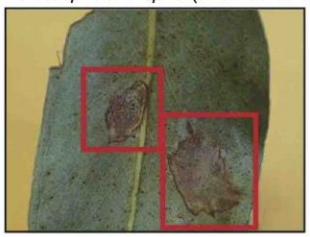


Teratosphaeria sp. 6

Teratosphaeria sp. 7 (following *Eurymeloides bicincta*)

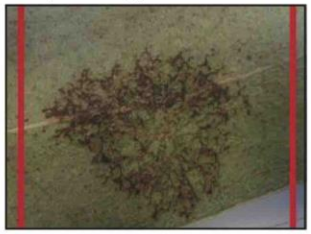


Teratosphaeria sp. 8 (and necrosis)



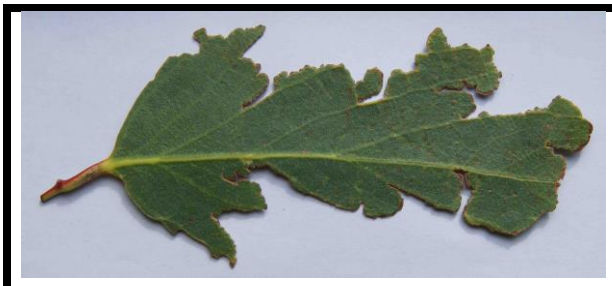
Teratosphaeria sp. 9 (early stage)

*Teratosphaeria sp.*10 (linked subepidermally)



Uraba lugens (eggs)

Fig. 2.S2. Photographs of causal organism symptoms (left) and a list of observed organisms involved (right) on *E. pauciflora* foliage from both Dungrove and Grassy Hut sites combined. Symptom are presented in alphabetical order.



***Cadmus spp.*:**

Cadmus australis

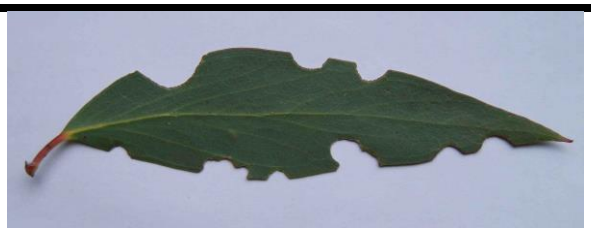
Cadmus crucicollis

Cadmus sp. (unknown)

Doratifera oxyeli

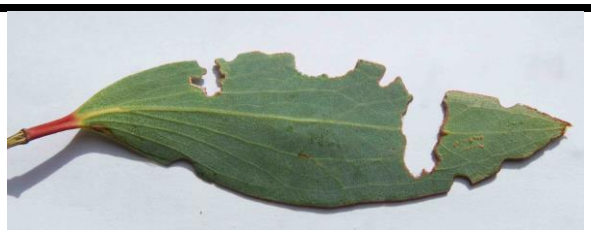
***Lepidoptera* (larvae):**

Opodiphthera helena



***Paropsisterna spp.*:**

Paropsisterna aegrota ellioti
Paropsisterna. agricola
Paropsisterna bimaculata
Paropsisterna decolorata
Paropsisterna laesa
Paropsisterna nobilitata
Paropsisterna sp. (recognized but undescribed)
Paropsisterna sp. (unknown)
Paropsisterna tasmanica
Paropsisterna variicollis



***Paropsisterna spp.* (larvae):**

Paropsisterna aegrota ellioti
Paropsisterna. agricola
Paropsisterna bimaculata
Paropsisterna decolorata
Paropsisterna laesa
Paropsisterna nobilitata
Paropsisterna sp. (recognized but undescribed)
Paropsisterna sp. (unknown)
Paropsisterna tasmanica
Paropsisterna variicollis

***Teratosphaeria spp.*:**

Appendix B – Supplementary material for Chapter 3

Table 3.S1. Organism identifications and symptom descriptions.

Organism causing leaf damage	**Order**	**Description of Leaf Damage**
Diphucephala colaspidoides	Coleoptera	finely rasped epidermal cells
Gonipterus scutellatus species complex - failed feeding by larvae	Coleoptera	bright green rectangle of the size and shape of larval Gonipterus tracks but with the epidermis still intact
Gonipterus scutellatus species complex - feeding by larvae	Coleoptera	parallel-sided slots though the leaf, or with a transparent layer of cuticle on the other surface of the leaf remaining; the slot-like tracks vary in length and density; additionally, a pair of straight, brown, sclerotised lines extending from a slot up to 5mm across the lamina.
Paropsisterna agricola larva	Coleoptera	chewed area narrower at leaf margin then expanding irregularly
Paropsisterna sp. adult	Coleoptera	leaf margins appear 'scalloped'
Cecidomyiidae sp. 1	Diptera	pyramid-shaped galls on one surface of lamina connected to a more rounded protusion on the other surface; multiple but separate (average height 8mm)
Cecidomyiidae sp. 2	Diptera	single, more rounded pyramid-shaped gall on one surface of lamina connected to a rounded protrusion on the other surface; (average height 8mm)
Diptera sp. 2	Diptera	tiny, brown scattered lesions at leaf apex, not prominent
Diptera sp. 3	Diptera	tiny, brown lesions along leaf margin, in an indistinct row
Diptera sp. 4	Diptera	tiny, brown, scaly lesions along leaf margin, in two indistinct rows
Diptera sp. 5	Diptera	reddish, spherical galls (2mm), along midvien at apex of leaf
Fergusonina sp. 1	Diptera	large (to 10mm diameter), irregular, fleshy gall without distinct structure, along leaf margin
Hymenoptera sp. 5	Diptera	tiny, pointy, pimple-like lesion scattered on leaf
Aulographina eucalypti	Fungal	concentric, necrotic rings visible on only one side of lamina which may be purple-edged in winter; ascospores 2 cells
Cryptosporiosis eucalypti	Fungal	irregular, warty, reddish-brown, lesions
Fumago vagans	Fungal	black, sooty, fungus; secondary to damage from sap feeding insects.
Pachysacca samuelii	Fungal	connective tissue finely warty, not sclerotic, frequently with light margins
Sonderhenia eucalyptorum	Fungal	small purple spots, not, or hardly, elevated that become necrotic with age
Teratosphaeria spp.	Fungal	small to large irregular blotches which commonly coalesce; purple, red-brown and gray; visible on both surfaces of the lamina
Eurymelinae sp. 1	Hemiptera	small, reddish, linear slerotism of vein adjacent to midvein

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Organism causing leaf damage	Order	Description of Leaf Damage
Eurymelinae sp. 2	Hemiptera	small, reddish, sclerotic spots on veins (pierced for sap feeding)
Eurymelinae sp. 3	Hemiptera	small, reddish, sclerotic spots between veins (pierced for sap feeding)
Eurymelinae sp. 4	Hemiptera	small, reddish, sclerotic spots adjacent to mid vein on abaxial surface
Eurymeloides bicincta	Hemiptera	small, reddish, sclerotic spots along mid vein on abaxial surface
Eurymeloides bicincta eggs	Hemiptera	small, reddish, linear slerotism along midvein
Australopsylla sp.	Homoptera: Psyllidae	small (2mm) wrinkled spherical galls on adaxial surface with slight pit on abaxial surface containing a flat, white lerp
Creiis sp. 1	Homoptera: Psyllidae	transparent, leongate lerp, abaxial surface of lamina
Ctenarytaina eucalypti	Homoptera: Psyllidae	white,waxy secretion, reddening around vein pierced for feeding
Glycaspis sp. 1	Homoptera: Psyllidae	white domed lerp covered with strands of waxy secretions; sometimes black strands due to sooty mould
Hyalinaspis sp. 1	Homoptera: Psyllidae	transparent, clamshell-like lerp (3mm), with psyllid covered in loose, white strands
Hyalinaspis sp. 2	Homoptera: Psyllidae	psyllid with translucent, brown, clamshell-like lerp (average 5mm)
Hyalinaspis sp. 3	Homoptera: Psyllidae	Psyllid with yellow clamshell-like lerp (average 8 mm)
Hyalinaspis sp. 4	Homoptera: Psyllidae	Psyllid with white clamshell-like lerp containing 2 halves (average 5mm)
Hyalinaspis subfasciata	Homoptera: Psyllidae	psyllid with transparent clamshell-like lerp (average 9mm)
Lasiopsylla rotundipennis	Homoptera: Psyllidae	almost flat, white lerps, often in groups, large, approx 3-5mm
Schedotrioza multitudinea	Homoptera: Psyllidae	small (to 5mm diameter), irregular, fleshy, spherical galls; often joined making shape indistinct

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Organism causing leaf damage	Order	Description of Leaf Damage
Schedotrioza sp. 1	Homoptera: Psyllidae	mostly oval-shaped hole with brown margin of exposed mesophyll tissue (up to 4mm), regular distance from leaf margin
Schedotrioza sp. 3	Homoptera: Psyllidae	slightly elliptical gall on abaxial midvein of leaf; approx 3mm
Schedotrioza sp. 4	Homoptera: Psyllidae	tiny gall (approx 1.5mm) at base of leaf, on midvein, frequently with erupted opening
Schedotrioza sp. 5	Homoptera: Psyllidae	tiny gall(1mm) near abaxial margin of leaf, no syptom on adaxial surface
Schedotrioza sp. 6	Homoptera: Psyllidae	gall causing lamina to bulge on one side leaving a small pit on the other side
Hymenoptera sp 2	Hymenoptera	straight line of pimple-like lesions along margin of leaf
Hymenoptera sp. 6	Hymenoptera	tiny pimple-like lesions forming a ring approx 4mm diameter
Hymenoptera sp. 7	Hymenoptera	circular gall with irregular surface, some sclerotisation; not visible on other surface of lamina
Hymenoptera sp. 8	Hymenoptera	separate but numerous sclerotic, pimple-like galls across lamina (< 1mm diameter),
Ophelimus eucalypti	Hymenoptera	spherical galls 3-5mm embedded in leaf, prodruding and visible from both surfaces; often with a purplish hue
Phylacteophaga froggatti	Hymenoptera : Pergidae	broad, flat, transparent blister of epidermal cells, frequently covering the entire abaxial lamina
Acrocercops laciniella	Lepidoptera	an irregular, transparent trail or, in later stages, blister of epidermal cells remaining after 'leaf mining' by the larvae which consumes mesophyll tissue
Agriophara gravis	Lepidoptera	a dead leaf joined by web to a live one to provides a shelter for the caterpillar which grazes on surface tissue
Heliozela prodela	Lepidoptera	shot hole with smooth edges up to 4mm
Heliozela sp. 2	Lepidoptera	brown rectangular blister on adaxial lamina with an exit hole at one end on the abaxial surface
Hemibela heliotricha	Lepidoptera	5-10mm twig upright on mid-vein, and standing perpendicular to lamina. Twig hollow to provide shelter for a larva
Hyalarcta sp. 1	Lepidoptera	small (up to 5mm) brown patches of epidermis deeply grazed between veins but not penetrating the other laminal surface
Hypertropha tortriciformis	Lepidoptera	entire margin of leaf rolled and loosely webbed together; veins around which feeding occurs become quite dark

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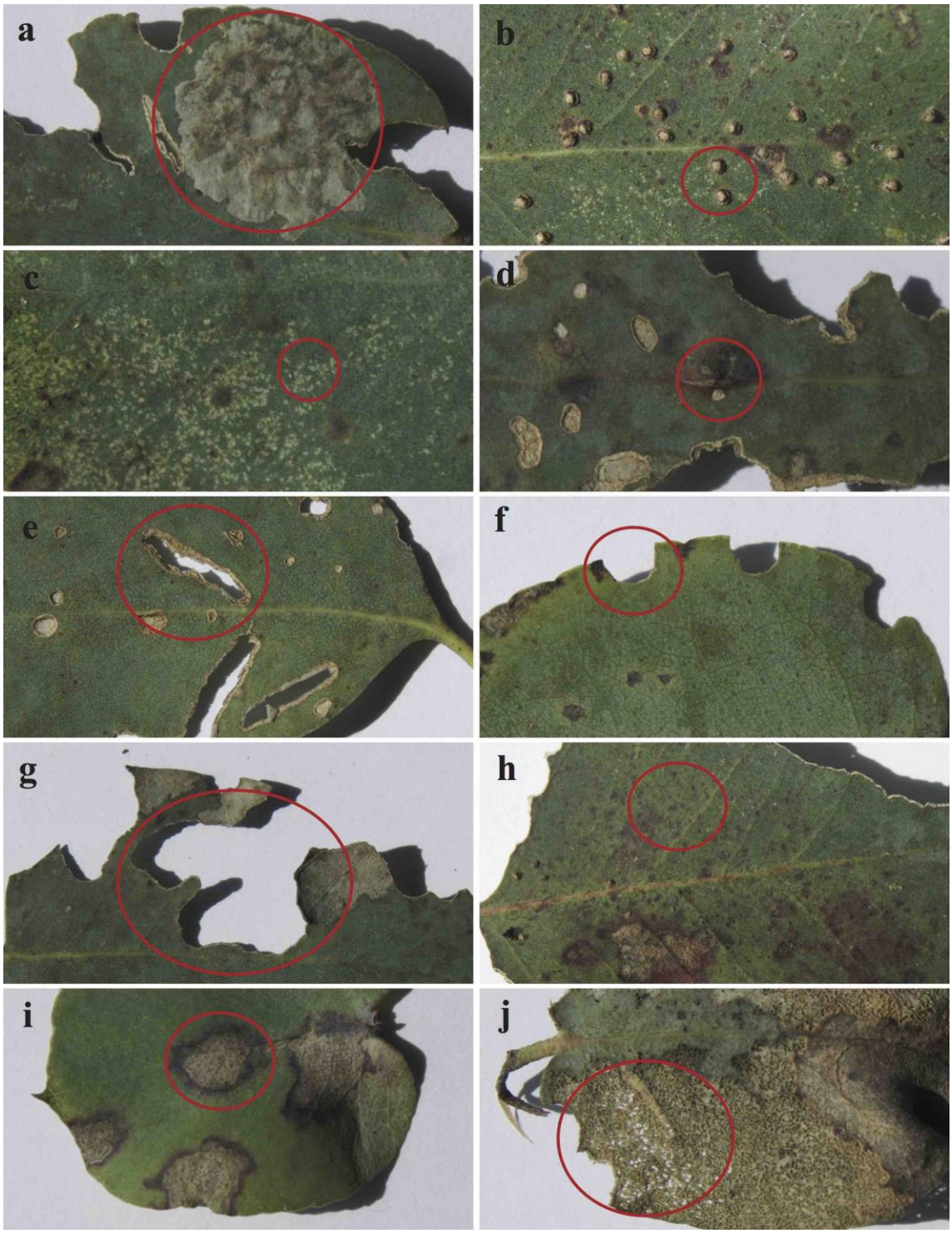
Organism causing leaf damage	**Order**	**Description of Leaf Damage**
Mnesampela privata	Lepidoptera	leaves at tips of branches loosely webbed together; tracks of grazed parenchyma become dark brown
Oecophoridae sp. 1	Lepidoptera	three live leaves strongly held closely together with silk
Tortricidae sp. 1	Lepidoptera	margin of leaf rolled and held with parrallell strands of silk
Uraba lugens	Lepidoptera	skeletonised i.e. only veins are visible following grazing of parenchyma
Unknown 1		regular inverse-scalloping along leaf margin
Unknown 2		herbivory of leaf margin seems to have occurred when the leaf was young and expanding, resulting in distortion so it is difficult to assign a specific organism to the cause
Unknown 3		tear-shaped holes adjacent to midvein; appox 5mm long
Unknown 4		brown lesion forming a straight line extending up to 10mm
Unknown 6		similar straight line lesion as unknown 4, but parallel pairs of lesions approx. 3mm apart

Fig. 3.S1. Photographs of select causal organism symptoms on *E. morrisbyi* foliage (See Table S1 for descriptions). (a) *Acrocercops laciniella*, (b) *Aulographina eucalypti*, (c) *Diphucephala colaspidoides*, (d) *Eurymeloides bicincta* (eggs), (e) *Gonipterus scuttelatus* (larvae), (f) *Paropsisterna spp*., (g) *Paropsisterna agricola* (larvae), (h) *Sonderhenia eucalyptorum*, (i) *Teratosphaera spp*., and (j*) Uraba lugens*. Red circles highlight damage types.



Appendix C – Supplementary material for Chapter 4

Table 4.S1. Putative eucalypt orthologs of key genes involved in wax biosynthesis and secretion in *Arabidopsis*, their position in the *Eucalyptus grandis* genome and the linkage map used in this study.

Gene[1]	TAIR accession	Ref[2]	*E. grandis* v1.1 locus	Position (v1.1)[3]	LG	cM
Biosynthesis						
FATB	At1g08510	1	Eucgr.C01082	3: 17,073,720..17,078,776	3	39.6
FATB	At1g08510	1	Eucgr.C04139	3: 76,199,729..76,204,951	3	116.3
CER10	At3g55360	1	Eucgr.G02931	7: 47,960,182..47,963,539	7	99.8
CER4	At4g33790	1	Eucgr.J00479	10: 5149162..5155037	10	11.3
KCS11	At2g26640	2	Eucgr.H00225	8: 2,517,118..2,519,921	8	1.0
WBC11	At1g17840	1	Eucgr.H00674	8: 8,997,407..9,001,960	8	14.3
Secretion						
CER7	At3g60500	1	Eucgr.H02323	8: 31,156,901..31,163,901	8	56.9
CER2	At4g24510	1	Eucgr.F00422	6: 5,365,081..5,369,447	6	17.1
CER2	At4g24510	1	Eucgr.F00425	6: 5,395,042..5,398,390	6	17.1
CER3/WAX2/YRE/FLP1	At5g57800	1	Eucgr.C00214	3: 4,750,456..4,755,769	3	2.5
Regulation						
WIN1/SHN1	At1g15360	3	Eucgr.C01178	3: 18,251,536..18,253,293	3	42.0
WIN1/SHN1	At1g15360	3	Eucgr.C04221	3: 77,338,372..77,339,426	3	117.4
CFL	At2g33510	3	Eucgr.H03380	8: 49,477,993..49,480,972	8	85.2
HDG1	At3g61150	3	Eucgr.K00234	11: 2910251 - 2914337	11	0.0
WAR3/RDR1	A11g14790	3	Eucgr.B02681	2: 49,390,132..49,393,116	2	91.6
WAR3/RDR1	At1g14790	3	Eucgr.G02092	7: 38,367,587..38,372,609	7	52.4
WAR4/SGS3	At5g23570	3	Eucgr.H05056	8: 72360633 - 72364511	8	134.0
CER9	At4g34100	3	Eucgr.I01130	9: 22197483 - 22203850	9	35.1

[1]Genes names follow the source reference. Where a locus has multiple names, only the first is reported in figure 4 and the manuscript text. In several cases, multiple eucalypt homologs were detected for a single *Arabidopsis* locus.

[2]1 = Samuels *et al.* (2008); 2 = Lokesh *et al.* (2013); 3 = Lee and Suh (2013).

[3](scaffold: position in base pairs).

Table 4.S2. Genotype means at the QTL for cuticular wax compounds.

Compound	L.G.[a]	Nearest marker[b]	Map pos (cM)	Parental genotypes[c] ♀	♂	Seg[d]	Genotype means[e]			
Aliphatic esters										
Benzyl n-eicosanoate (C20)	6	p03b10	83.3	nn	np	M p10b11	nn 0.001	np 0.003		
Benzyl n-docosanoate (C22)	1	Emb56	3.5	ab	cd	B	ac 0.011	ad 0.011	bc 0.011	bd 0.004
	8	599919	1.0	nn	np	M	nn 0.012	np 0.005		
Benzyl n-tetracosanoate (C24)	1	Emb56	3.5	ab	cd	B	ac 0.075	ad 0.064	bc 0.086	bd 0.031
	3	567811	109.8	lm	ll	F 503607	lm 0.078	ll 0.045		
	8	599919	1.0	nn	np	M	nn 0.095	np 0.024		
Benzyl n-hexacosanoate (C26)	8	599919	1.0	nn	np	M	nn 0.126	np 0.057		
Benzyl n-octacosanoate (C28)	3	642858	30.2	nn	np	M 641603	nn 0.136	np 0.113		
	6	573332	122.2	nn	np	M 573315	nn 0.134	np 0.114		
	8	503944	134.6	lm	ll	F	lm 0.114	ll 0.144		
Phenylethyl n-eicosanoate (C20)	3	642858	30.2	nn	np	M 641603	nn 0.007	np 0.003		
	3	564559	66.3	lm	ll	B	lm 0.004	ll 0.007		
	5	575337	0.1	nn	np	M	nn 0.006	np 0.004		
	6	565210	101.4	ab	cd	B Emb173	ac 0.004	ad 0.007	bc 0.004	bd 0.004
Phenylethyl n-docosanoate (C22)	3	569486	82.7	lm	ll	B	lm 0.003	ll 0.005		
	5	p02b02	63.8	lm	ll	F	lm 0.003	ll 0.005		
	7	574475	99.0	nn	np	M 504269	nn 0.005	np 0.003		
	9	p10b04	90.6	ef	eg	B Emb204	ee 0.005	ef 0.003	eg 0.004	eg 0.004
Phenylethyl n-tetracosanoate (C24)	3	p01b01	86.0	nn	np	B	nn 0.007	np 0.010		
	8	599919	1.0	nn	np	M	nn 0.012	np 0.006		
Phenylethyl n-pentacosanoate (C25)	8	599919	1.0	nn	np	M	nn 0.001	np 0.000		
Phenylethyl n-hexacosanoate (C26)	3	p01b01	86.0	nn	np	B	nn 0.008	np 0.015		
	9	p10b04	90.6	lm	ll	F 503689	lm 0.010	ll 0.014		
Phenylethyl n-octacosanoate (C28)	3	p01b01	86.0	nn	np	B	nn 0.014	np 0.034		
	10	562745	4.9			-				
Aliphatic β-diketones										
n-Hetriacontane-14, 16-dione (C31)	8	503660	19.5	nn	np	M 566660	nn 1.340	np 0.878		
	11	CRC2	77.9	nn	np	B	nn 0.958	np 1.206		
n-Tritriacontane-16, 18-dione (C33)	1	639448	73.0	nn	np	M 565878	nn 9.850	np 10.734		
	8	p11b07	35.7	nn	np	M	nn 9.691	np 10.978		
	11	571276	64.2	lm	ll	B	lm 9.763	ll 10.873		
Hydrocarbons										
n-Nonacosane	11	562840	54.9	nn	np	B	nn 0.222	np 0.178		

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Compound	L.G.[a]	Nearest marker[b]	Map pos (cM)	Parental genotypes[c] ♀	♂	Seg[d]	Genotype means[e]			
Ketones										
Heptadecanone	1	567923	11.7	hk	hk	M	kk 0.165	h- 0.154		
	8	566660	15.2	nn	np	M	nn 0.160	np 0.153		
	11	571397	63.6	nn	np	B	nn 0.151	np 0.161		
Aldehydes										
Hexadecanal	3	571717	15.0	ef	eg	B Emb115	ee 0.541	ef 0.479	eg 0.470	fg 0.482
	6	Emb173	89.8	ab	cd	F	ac 0.542	ad 0.520	bc 0.447	bd 0.477
	10	570364	6.3	lm	ll	F 639769	lm 0.480	ll 0.506		
Total wax	1	Emb12	68.1							
	3	CSA_1872	4.7							
	8	573772	117.8							
	11	571276	64.2							
Flavonoids										
Desmethyl eucalyptin	1	565878	64.4	nn	np	M	nn 0.442	np 0.385		
	2	575243	0.0	lm	ll	F	lm 0.389	ll 0.429		
	3	640146	120.3	nn	np	B	nn 0.435	np 0.391		
	5	p02b02	63.8			-				
	8	562846	23.8	nn	np	M	nn	nn 0.463	np 0.365	
	10	Emb155	101.3	ef	eg	B	Ee 0.377	Ef 0.450	Eg 0.425	0.395
	11	575083	75.9	nn	np	M CRC2	nn 0.362	np 0.451		
Eucalyptin	1	Emb180	59.5	ef	eg	M	ee 0.290	ef 0.342	eg 0.454	fg 0.477
	5	571218	66.30			-				
***Mnesampela private* (AGM)**	3	642858	30.2	lm	ll	B	lm 1.762	ll 1.511		
	8	599919	1.0	nn	np	M	nn 1.379	np 1.925		

[a] Linkage group.

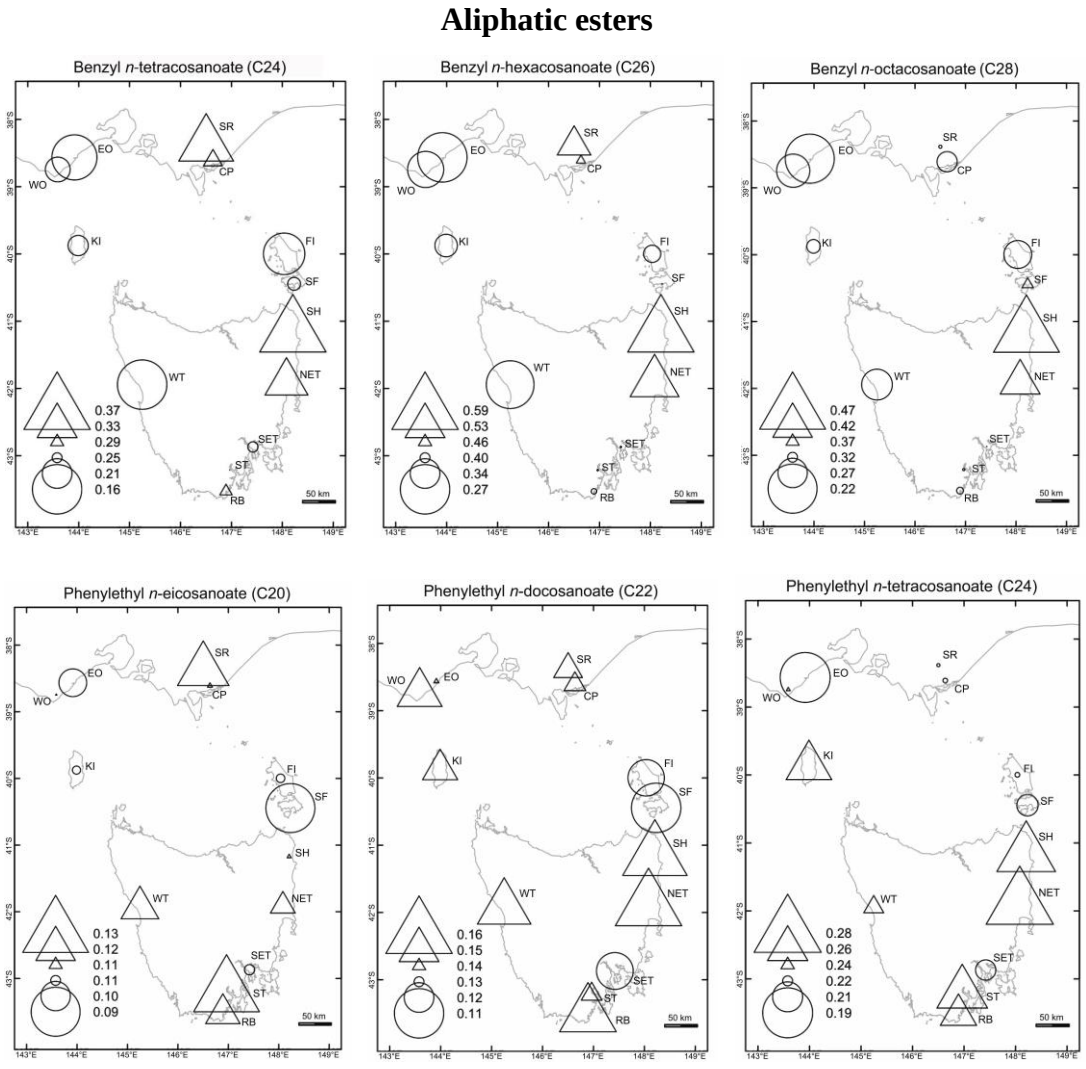
[b] Markers names beginning with Emb or CRC are microsatellites, those named p#b# are AFLP, while the remainder are DArT.

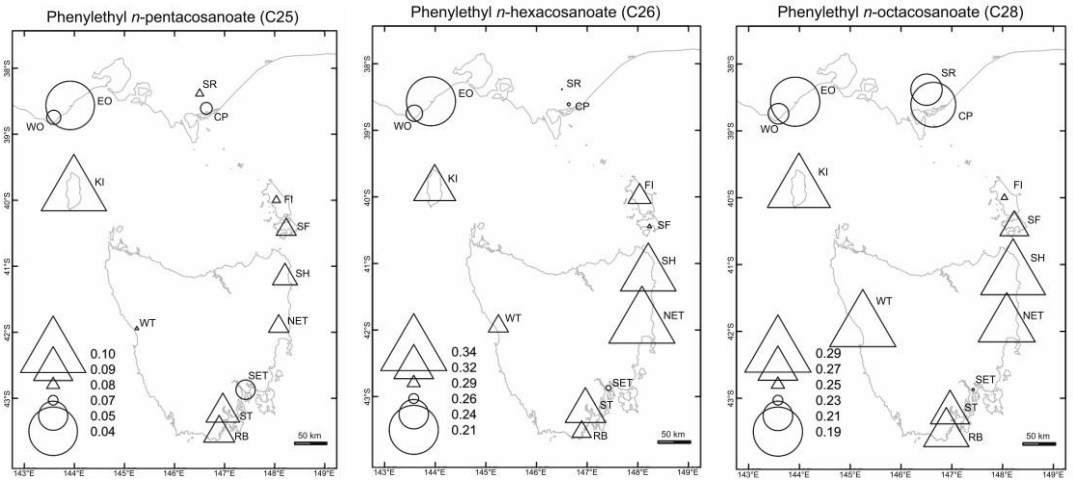
[c] Genotype coding for 'parental genotypes' and 'genotype means' follows the coding required for MAPQTL 6.0 (Van Ooijen 2009).

[d] Indicates whether segregation of the QTL effect is from the male parent (M), the female parent (F), or both (B). For QTL where the effect segregated solely from one parent, if the marker nearest to the QTL peak was not segregating from the same parent as the QTL effect, the nearest marker segregating from the same parent as the QTL is shown in this column, and the parental genotypes and genotype means at this marker are given in the columns proceeding and following. Where the QTL effect segregated from both parents, the parental genotypes and genotype means at a marker segregating from both parents is shown, if present close to the QTL peak.

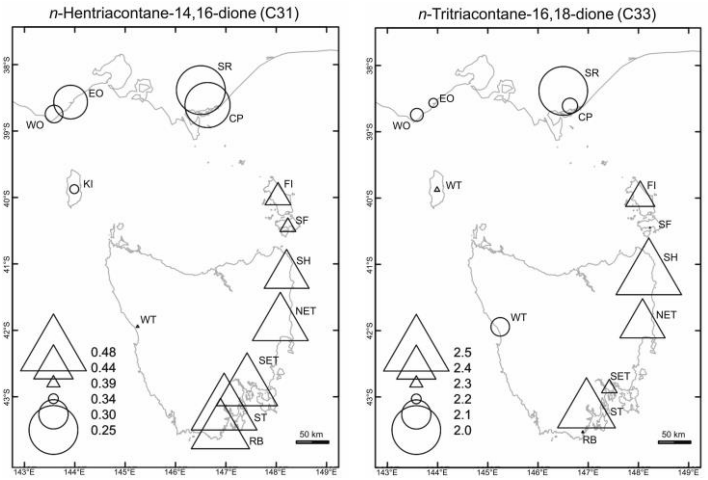
[e] All compounds were expressed as $mg.g^{-1}DM$

Fig. 4.S1. Genetic variation in cuticular wax compounds between geographic sub-races of *Eucalyptus globulus* in a common garden trial. Values are the least square means obtained from the mixed-model analysis of individual compound concentrations (mg/gDM). *Triangles* indicate higher values with larger triangles representing the highest values. *Circles* indicate lower values with larger circles representing the lowest values. Sub-race codes are as follows: (CP) Coastal Plain, (EO) Eastern Otways, (FI) Flinders Island, (KI) King Island, (NET) Northeastern Tasmania, (RB) Recherche Bay, (SET) South-eastern Tasmania, (SF) Southern Furneaux, (ST) Southern Tasmania, (SH) St. Helens, (SR) Strzelecki Ranges, (WO) Western Otways, and (WT) Western Tasmania.

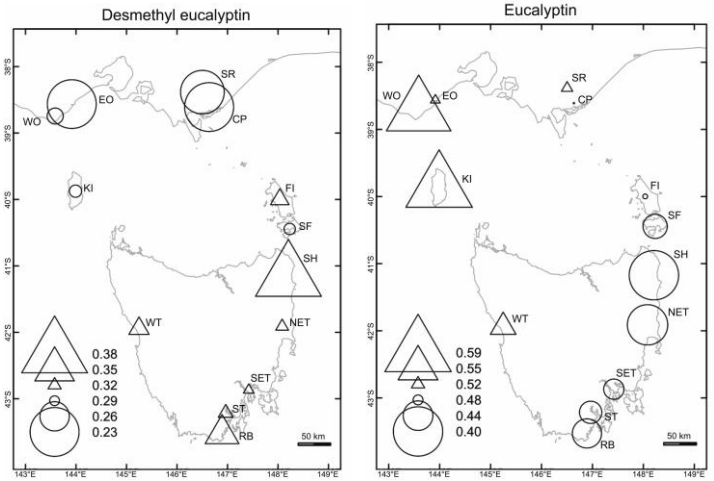




Aliphatic β-diketones

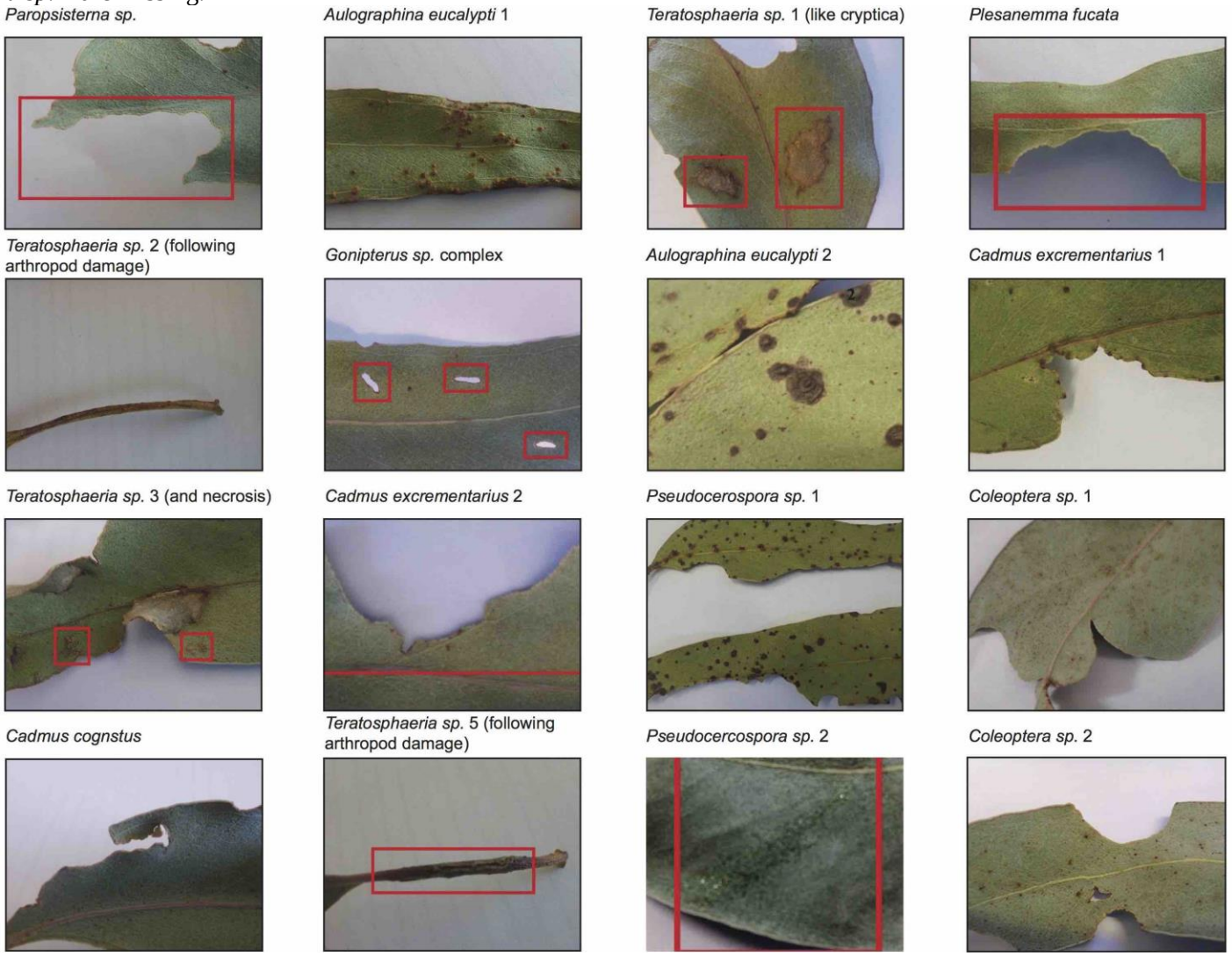


Flavonoids



Appendix D – Supplementary material for Chapter 5

Fig. 5.S1. Photographs of causal organism symptoms on *E. globulus* foliage. Symptoms are presented in order of their average proportional abundance per tree from highest to lowest (going left to right) in each row of photographs. Only photographs for symptoms of *Teratosphaera sp.* 4 and *Coleoptera sp.* 4 are missing.



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Coleoptera sp. 3

Ophelimus sp. & *Myllorhinus dentifer*

Glycaspis cameloides (1st instar)

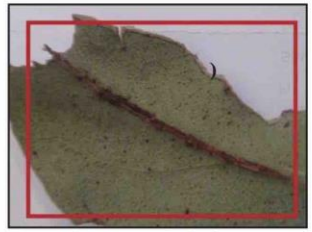
Lepidoptera: Psychidae

Teratosphaeria sp. 6

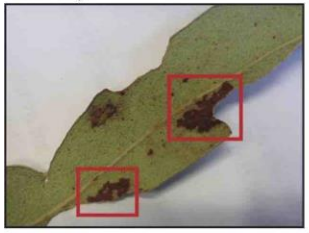
Teratosphaeria cryptica (early stage)

Pseudocercospora sp. 3

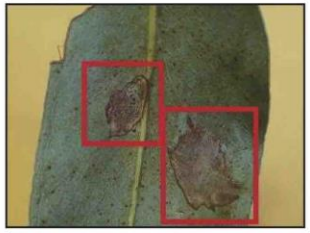
Teratosphaeria sp. 7 (following *Eurymeloides bicincta*)



Lepidoptera grazing (followed by necrosis)



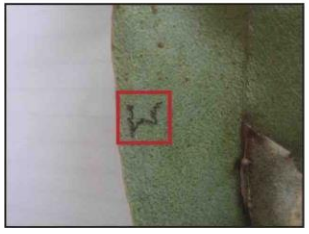
Teratosphaeria sp. 8 (and necrosis)



Hymenoptera sp.

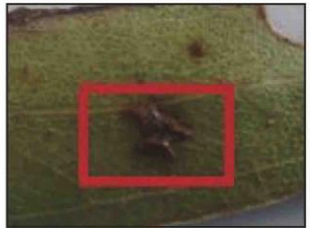
Paropsisterna agricola (egg cases and larval moult)

Lepidoptera: Leaf miner



Acari sp. 1

Pachysacca samuelii



Teratosphaeria sp. 9 (early stage)

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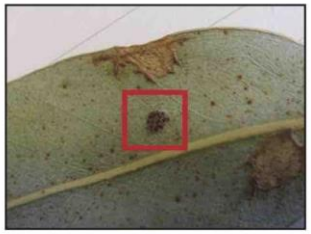
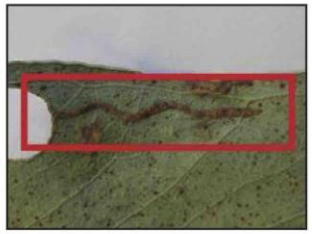
Acrocercops laciniella

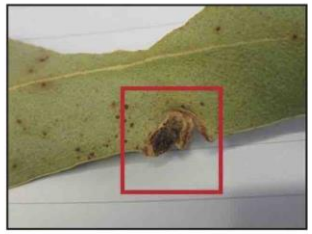
Acari sp. 2



Nepticulidae sp.



Schedotrioza sp.



Teratosphaeria sp. 10 (linked subepidermally)

Appendix E - Supplementary material for Chapter 6

Table 6.S1. Symptom identifications and descriptions

Symptom name[a]	Guild[b]	QTL detected[c]	Description of symptom damage
Aulographina eucalypti	F	Yes	
Seridium eucalypti	F	Yes	stem canker
Fungi 1	F	Yes	tan dots
Fungi 2	F	Yes	small tan circle with black dots
Fungi 3	F	Yes	mid-vein crack
Fungi 4	F	No	margin vain crack
Fungi 5	F	No	vein crack
Cadmus spp.	C	Yes	
Doratifera oxleyi	C	Yes	tip
Gonipterus scutellatus	C	Yes	weevil larvae
Heliotrioza	C	Yes	shot holes
Hetereonyx	C	Yes	
Schedotioza	C	Yes	small holes near margin
Chewer 1	C	Yes	irregular holes, communal feeding early instar chrysomelid
Chewer 2	C	Yes	chrysomelid or weevil adult scalloping
Chewer 3	C	Yes	rough
Chewer 4	C	Yes	smooth
Chewer 5	C	No	split/divided leaf tip
Paropsisterna variicolis	C	No	1st instar
Diphucephalus spp.	C	No	tearing/jagged
Chewer 8	C	No	smooth stepped tip
Chewer 9	C	No	straight slot
Agriophara sp.	G	No	
Aterpus rubus	G	Yes	weevil grazed bark
Mnesampela private	G	Yes	
Oecophoridae 1	G	Yes	
Oecophoridae 2	G	Yes	watery patch
Grazer 1	G	Yes	surface graze jigsaw
Perthida spp.	G	No	Jarrah leaf miner
Grazer 2	G	No	*Oecophoridae* with ridge
Grazer 3	G	No	Skeletonizing with big holes – unknown
Uraba lugens	G	No	Skeletonizing
Grazer 5	G	No	surface miner
Acrocercops sp.	G	No	larvae
Grazer 7	G	No	deep graze with white crescent tube
Grazer 8	G	No	surface deep graze, scratchy, small area
Grazer 9	G	No	fragile pale margin blister with hole
Ophelimus spp.	S	Yes	wasp gall on petiole
Phylacteophaga forggatti	S	Yes	smooth brown blister wasp, often grazing next to it
Sap Sucker 1	S	Yes	bump on leaf, hollow under
Sap Sucker 2	S	Yes	pyramid gall through leaf, pointy
Sap Sucker 3	S	Yes	spherical gal on twig
Sap Sucker 4	S	Yes	wasp twig bumps
Sap Sucker 5	S	No	sphere on leaf pit under
Sap Sucker 6	S	No	spherical gall through leaf (margin or vain) 5mm diam
Sap Sucker 7	S	No	strawberry bump, thumb-sized, hollow under
Sap Sucker 8	S	No	wasp stem gall
Sap Sucker 9	S	No	curved/spiral leaf
Predator 1	P	Yes	fluffy web joining leaves
Predator 2	P	No	

[a] Organism causing the damage.

[b] Damage type guild for individual symptoms consisting of fungal pathogens (F), leaf chewers (C), leaf grazers (G), sap suckers (S) and predators (P).

[c] Whether or not QTL were detected for the individual symptom.

Table 6.S2. Genotype means at the QTL for community-level traits and individual community members in *Eucalyptus globulus*.

Trait	Mean[a]	sd[b]	clonal repeatability[c]	LG[d]	Map pos[e]	Peak LOD[f]	Nearest Marker[g]	Parental genotype[h] ♀	Parental genotype[h] ♂	Seg.[i]	Genotype means			
Community-level traits														
NMDS1	-	-	0.12 ± 0.12	2	53.4	3.0	Emb158	ef	eg	F	ee -0.0285	ef 0.0148	eg 0.0723	fg -0.0684
				5	59.4	4.8*	570670	lm	ll	F	lm -0.0567	ll 0.0242		
				8	92.0	3.2	568780	hk	hk	F	kk 0.0607	h- -0.0237		
				11	61.9	7.7***	571397	nn	np	M	nn -0.0728	np 0.0325		
NMDS2	-	-	0.25 ± 0.14	9	11.2	3.5	640225	nn	np	M	nn -0.0248	np -0.0769		
NMDS3	-	-	0.22 ± 0.13	2	104.4	3.6	CRC8	ab	cd	M	ac -0.0268	ad -0.0427	bc -0.0478	bd 0.0636
				5	87.8	6.2***	Emb208	lm	ll	F	lm 0.0271	ll -0.0795		
				7	23.3	4.3*	566500	lm	ll	F	lm 0.0300	ll -0.0733		
				10	80.9	3.5	644030	hk	hk	M	kk -0.0858	h- 0.0033		
Abundance	233.41	47.89	0.03 ± 0.09	2	53.4	4.9*	Emb158	ef	eg	F	ee 232.321	ef 237.620	eg 204.538	fg 252.508
				6	59.4	4.0	CRC11	ab	cd	B	ac 220.680	ad 238.383	bc 231.682	bd 270.51
				9	8.2	4.7*	564698	hk	hk	M	kk 218.812	h- 243.686		
Richness	16.10	2.56	0.09 ± 0.11	5	81.4	5.4**	568743	lm	ll	F	lm 15.410	ll 16.4545		
				11	52.2	3.7	562840	nn	np	M	nn 15.638	np 16.250		
Evenness	0.62	0.05	0.13 ± 0.13	1	70.0	3.4	Emb12	ab	cd	F	ac 0.59720	ad 0.62520	bc 0.61400	bd 0.6178
Individual symptoms														
Aulographina eucalypti	50.23	27.19	0.27 ± 0.13	3	111.6	3.4	563549	nn	np	M	nn 61.500	np 41.662		
				7	14.4	3.3	p14b03	nn	np	M	nn 58.973	np 44.057		
Seridium eucalypti	1.00	0.79	0.22 ± 0.14	9	0.0	3.6	575650	nn	np	M	nn 1.111	np 0.8826		
				11	63.5	6.3***	571397	nn	np	F	nn 1.333	np 0.7500		
Fungi 1	1.57	2.29	0.15 ± 0.12	8	135.6	3.5	503944	lm	ll	F	lm 1.2227	ll 2.28846		
Fungi 2	0.12	0.31	0.31 ± 0.14	4	54.1	3.8	641176	nn	np	M	nn 0.0974	np 0.2205		
Fungi 3	1.42	1.47	0.13 ± 0.12	2	0.0	3.5	575243	lm	ll	F	lm 1.4342	ll 1.38182		
				3	109.5	3.7	567811	nn	np	M	nn 1.7865	np 1.0896		
Cadmus spp.	3.84	4.58	0.32 ± 0.13	5	52.8	5.2	p06b11	nn	np	M	nn 4.1800	np 2.4945		
				10	56.7	3.2	*COBL4*	lm	ll	F	lm 2.9351	ll 3.91071		
Doratifera oxleyi	54.07	20.73	0.07 ± 0.11	6	59.4	4.2	CRC11	ab	cd	M	ac 49.6500	ad 57.4000	bc 54.4167	bd 71.900
Gonipterus scutellatus (sqrt)	7.03	6.00	0.00 ± 0.09	1	95.8	3.4	644046	lm	ll	B	lm 2.4816	ll 2.36924		
				11	59.9	4.4	571397	nn	np	M	nn 2.2324	np 2.5622		
Heliotrioza	0.08	0.32	0.00 ± 0.08	4	35.3	4.0	p13b15	lm	ll	F	lm 0.0550	ll 0.1607		
Hetereonyx	0.08	0.79	-	11	28.5	4.2	p15b02	nn	np	M	nn 0.0102	np 0.5434		
Schedotrioza	0.08	0.32	0.00 ± 0.09	2	61.2	4.4	568300	lm	ll	F	lm 0.0555	ll 0.0223		
				9	12.2	6.1	p15b01	nn	np	M	nn 0.0153	np 0.0815		
Chewer 1	0.32	0.72	0.07 ± 0.11	5	19.1	3.2	p17b05	nn	np	M	nn 0.4655	np 0.2272		
Chewer 2	80.97	19.30	0.00 ± 0.08	8	120.8	3.2	503819	nn	np	B	nn 82.345	np 87.355		
				9	86.1	5.7*	571807	nn	np	M	nn 80.880	np 88.845		
Chewer 3	14.09	11.50	0.36 ± 0.12	7	25.2	5.3**	566500	lm	ll	F	lm 13.402	ll 8.16667		
				10	65.2	3.1	566988	lm	ll	F	lm 8.0450	ll 13.3547		

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Trait	Mean[a]	sd[b]	clonal repeatability[c]	LG[d]	Map pos[e]	Peak LOD[f]	Nearest Marker[g]	Parental genotype[h] ♀	Parental genotype[h] ♂	Seg.[i]	Genotype means			
Chewer 4	6.07	5.33	0.07 ± 0.11	1	69.0	5.6*	Emb12	ab	cd	B	ac 3.75000	ad 8.30000	bc 5.95000	bd 4.8157
				3	10.7	3.5	644451	lm	ll	B	lm 4.5904	ll 6.50543		
				11	26.5	8.3*	p15b02	nn	np	M	nn 4.6173	np 6.7989		
Mnesampela privata	0.04	0.17	0.00 ± 0.09	9	45.1	5.1	642464	lm	ll	F	lm 0.0367	ll 0.04583		
Oecophoridae 1 (sqrt)	2.40	2.57	0.32 ± 0.13	1	24.9	3.8	640754	nn	np	M	nn 1.3359	np 1.3946		
				8	15.2	4.3*	566660	nn	np	M	nn 1.5061	np 1.2340		
Oecophoridae 2	0.08	0.28	0.00 ± 0.09	8	68.2	3.3	p10b03	nn	np	B	nn 0.0875	np 0.0754		
Grazer 1	0.14	0.93	0.00 ± 0.08	3	4.7	3.1	*CSA3*	hk	hk	M	hh 0.000	hk 0.3977	kk 0.0000	
				11	10.9	4.5	503068	nn	np	M	nn 0.3684	np 0.0625		
Aterpus rubus	0.75	0.91	0.01 ± 0.09	2	115.1	3.8	p03b02	nn	np	M	nn 0.4795	np 0.2606		
Ophelimus spp. (sqrt)	0.42	1.09	0.10 ± 0.11	4	21.0	4.0	Emb156	ab	cd	F	ac 0.3169	ad 0.62942	bc 0.34761	bd 0.2496
				5	2.1	7.2**	575337	nn	np	M	nn 0.5515	np 0.2245		
				6	28.1	6.6**	567551	lm	ll	F	lm 0.3428	ll 0.3724		
				8	15.2	9.2***	566660	nn	np	M	nn 0.2279	np 0.4707		
Phylacteophaga froggatti	3.27	3.44	0.00 ± 0.09	1	54.2	5.0*	Emb180	ef	eg	M	ee 2.28125	ef 4.08000	eg 4.94118	fg 2.54167
				3	10.7	3.1	644451	lm	ll	F	lm 3.1489	ll 3.81522		
				4	14.4	4.6*	Emb78	nn	np	B	nn 3.277	np 3.431		
Sap Sucker 2	0.08	0.23	0.00 ± 0.08	4	27.3	3.5	Emb156	ab	cd	F	ac 0.1111	ad 0.2058	bc 0.0000	bd 0.0434
Sap Sucker 3	0.04	0.22	0.00 ± 0.09	11	51.2	3.1	565569	nn	np	M	nn 0.0592	np 0.0000		
Sap Sucker 4	0.04	0.17	0.00 ± 0.09	2	12.0	9.1*	573524	hk	hk	F	kk 0.4166	h- 0.02960		
Sap Sucker 5	0.04	0.17	0.92 ± 0.02	2	12.0	16.5*	573524	hk	hk	F	kk 0.02777	h- 0.009868		
				8	86.3	3.2	565854	lm	ll	F	lm 0.0041	ll 0.03125		
Predator 1	0.01	0.08	0.42 ± 0.12	2	0.0	7.6	575243	lm	ll	F	lm 0.0000	ll 0.02272		
FPCs														
Macrocarpal G	2.50	0.90	0.51 ± 0.07	1	32.2	3.7	644199	nn	np	M	nn 6.3974	np 5.8275		
				6	125.1	14.4***	571558	lm	ll	F	ll 5.0819	lm 7.0256		
Sideroxylonal A (log)	6.07	1.49	0.79 ± 0.04	1	64.4	7.9***	565878	nn	np	M	nn 1.2674	np 1.1680		
				2	39.6	7.3***	638234	nn	np	M	nn 1.1307	np 1.2954		
				3	10.7	5.1**	644451	lm	ll	F	ll 1.1851	lm 1.2511		
				5	104.2	3.6	Emb37	ab	cd	F	ac 1.1900	ac 1.1717	bc 1.2408	bd 1.2591
				6	125.1	19.6***	571558	lm	ll	F	ll 1.3954	lm 1.3725		
				7	100.5	11.8***	574475	lm	ll	M	ll 1.1890	lm 1.2422		
				10	27.5	3.8	504254	nn	np	M	nn 1.1635	np 1.2723		
Terpenes														
Monoterpenes														
p-Cymene (sqrt)	0.01	0.00	0.10 ± 0.09	6	91.7	3.4	Emb173	ab	cd	F	ac 0.0070	ad 0.0046	bc 0.0053	bd 0.0057
				7	2.9	5.4**	Emb98	ef	eg	F	ee 0.0052	ef 0.0074	eg 0.0047	fg 0.0048
				9	64.8	4.7*	570860	hk	hk	F	kk 0.0057	h- 0.0055		
1_8-Cineole	6.05	2.35	0.03 ± 0.09	3	82.6	3.3	569486	lm	ll	B	ll 5.5303	lm 6.5649		
α-Terpineol (log)	0.02	0.01	0.60 ± 0.06	2	21.1	4.1	638739	lm	ll	F	ll 0.0206	lm 0.0209		
				11	71.1	4.3*	p16b03	nn	np	B	nn 0.0186	np 0.0223		

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Trait	Mean[a]	sd[b]	clonal repeatability[c]	LG[d]	Map pos[e]	Peak LOD[f]	Nearest Marker[g]	Parental genotype[h] ♀	Parental genotype[h] ♂	Seg.[i]	Genotype means			
Nerol (sqrt)	0.01	0.01	0.06 ± 0.09	9	46.1	11.7***	642464	lm	ll	F	ll 0.0118	lm 0.0164		
				11	77.8	13.6***	CRC2	nn	np	B	nn 0.0124	np 0.0144		
Terpinyl_acetate	0.43	0.50	0.79 ± 0.04	6	57.8	34.5***	CSRg62	ab	cd	M	ac 0.0036	ad 0.2009	bc 0.0041	bd 0.1861
Neryl acetate (log)	0.01	0.00	0.64 ± 0.06	3	9.4	4.1	Emb115	ef	eg	F	ee 0.0065	ef 0.0085	eg 0.0096	fg 0.0073
				6	67.7	8.2***	p16b11	lm	ll	F	ll 0.0103	lm 0.0063		
				8	123.5	4.8*	503819	nn	np	B	nn 0.0081	np 0.0084		
Sesquiterpenes														
Alloaromadendrene (log)	0.02	0.01	0.37 ± 0.08	6	124.2	12.6***	571558	lm	ll	F	ll 1.1690	lm 1.9482		
Aromadendrene (log)	1.57	0.61	0.74 ± 0.04	2	57.2	8.0***	p03b06	nn	np	B	nn 1.6902	np 1.4400		
				6	125.1	49.8***	571558	lm	ll	F	ll 1.1690	lm 1.9482		
				7	64.4	10.7***	CSRg61	ef	eg	M	ee 1.5842	ef 1.3505	eg 1.7539	fg 1.4577
Bicyclogermacrene	0.01	0.00	0.37 ± 0.08	6	124.2	28.8***	571558	lm	ll	F	ll 0.0054	lm 0.0073		
				7	85.2	11.8***	575285	hk	hk	F	kk 0.0056	h- 0.0066		
β-eudesmol	0.12	0.12	0.79 ± 0.04	2	12.0	13.1***	573524	hk	hk	F	kk 0.1410	h- 0.1041		
				6	125.1	66.7***	571558	lm	ll	F	ll 0.2292	lm 0.0067		
				8	85.3	3.2	565854	lm	ll	F	ll 0.1439	lm 0.0975		
Eudesmyl_acetate	0.68	0.47	0.50 ± 0.11	2	12.0	11.3***	573524	hk	hk	F	kk 0.0283	h- 0.0214		
				6	125.1	66.0***	571558	lm	ll	F	ll 0.0466	lm 0.0015		
Globulol (log)	1.42	0.43	0.83 ± 0.03	2	12.0	9.3***	573524	hk	hk	F	kk 1.3214	h- 1.4549		
				6	125.1	25.7***	571558	lm	ll	F	ll 1.0986	lm 1.7199		
				7	64.4	7.4***	CSRg61	ef	eg	M	ee 1.4404	ef 1.2742	eg 1.5245	fg 1.3669
				9	18.6	4.0	599794	nn	np	M	nn 1.4953	np 1.3508		
				10	63.6	3.9	p19b06	lm	ll	F	ll 1.4834	lm 1.3523		
Viridiflorol	0.03	0.01	0.54 ± 0.07	2	12.0	4.1	573524	hk	hk	F	kk 0.0343	h- 0.0353		
				2	55.4	3.9	p03b06	nn	np	M	nn 0.0370	np 0.0327		
				6	125.1	17.3***	571558	lm	ll	F	ll 0.0285	lm 0.0411		
				7	63.4	3.8	CSRg61	ef	eg	M	ee 0.0362	ef 0.0304	eg 0.0359	fg 0.0354
Total oils	12.68	3.36	0.21 ± 0.09	6	93.7	3.6	Emb173	ab	cd	F	ac 13.3562	ad 11.4932	bc 13.3788	bd 13.0992

[a] Grand means for each trait.

[b] Standard deviation from the mean for each trait.

[c] Clonal repeatability was calculated fitting genotype as a random term in a linear mixed effect model for each community-trait.

[d] Linkage group

[e] Peak position of QTL.

[f] Peak LOD score for each QTL. Genome-wide significance is indicated by: *, p<0.05; **, p<0.01; ***, p<0.001. The remainder were significant at the suggestive level (chromosome-wide type I error rate <0.05).

[g] Marker names beginning with Emb or CRC are microsatellites, those named p#b# are AFLP, *COBL4* and *CSA3* are candidate genes used in a previous QTL study for wood properties (Freeman *et al.* 2013) and the remainder are DArT.

[h] Genotype coding for 'parental genotypes' and 'genotype means' follows the coding required for MAPQTL 6.0 (Van Ooijen 2009).

Note: Grand means, standard deviations and clonal repeatability estimates for FPCs and terpenes are those first reported in O'Reilly-Wapstra *et al.* (2011) and Freeman *et al.* (2008a), respectively, and are expressed as $mg/g^{-1}DM$.